COBAS AMPLICOR™

Roche Diagnostics

COBAS AMPLICOR™ Hepatitis C Virus Test, version 2.0

HCV

FOR *IN VITRO* DIAGNOSTIC USE.

Order Information

AMPLICOR® HCV Specimen Preparation Kit, version 2.0	HCV PREP	96 Tests	P/N: 21111086 ART: 11 1108 6 US: 83126
AMPLICOR HCV Controls Kit, version 2.0	HCV CTL	8 Sets	P/N: 21111175 ART: 11 1117 5 US: 83131
AMPLICOR HCV Amplification Kit, version 2.0	HCV AMP	96 Tests	P/N: 21111094 ART: 11 1109 4 US: 83127
COBAS AMPLICOR HCV Detection Kit, version 2.0	HCV DK	100 Tests	P/N: 21111132 ART: 11 1113 2 US: 83130
COBAS AMPLICOR Detection Reagents Kit	DK	100 Tests	P/N: 20757470 ART: 07 5747 0 US: 83276
COBAS AMPLICOR Conjugate Detection Reagent	CN4	200 Tests	P/N: 20764213 ART: 07 6421 3 US: 83305
COBAS AMPLICOR Wash Buffer	WB	500 Tests	P/N: 20759899 ART: 07 5989 9 US: 83314
COBAS AMPLICOR Internal Control Detection Kit	IC DK	100 Tests	P/N: 20757608 ART: 07 5760 8 US: 83281

P/N: 3138151 (018)

Intended Use

The COBAS AMPLICOR Hepatitis C Virus (HCV) Test, version 2.0 (v2.0) is an *in vitro* diagnostic, nucleic-acid amplification test for qualitative detection of HCV RNA in human serum or plasma from blood collected in EDTA (EDTA plasma). This test detects by reverse-transcribing target HCV RNA into complementary DNA (cDNA), amplifying cDNA by polymerase chain reaction (PCR), hybridizing amplified cDNA with an oligonucleotide probe that binds enzyme, and catalyzing conversion of substrate to a colored product that is recognized by the COBAS AMPLICOR™ Analyzer. The COBAS AMPLICOR HCV Test, v2.0 is indicated for patients who have evidence of liver disease and antibody evidence of HCV infection, and who are suspected to be actively infected with HCV. Detection of HCV RNA indicates that the virus is replicating and therefore is evidence of active HCV infection.

Warnings:

1. Detection of HCV RNA, by itself, does not:
   - distinguish between acute and chronic states of infection
   - indicate the presence of liver disease.
2. A Positive result should be interpreted with caution in a patient who does not have antibody evidence of HCV infection (not tested, non-reactive by anti-HCV enzyme immunoassay or negative by anti-HCV strip immunoassay).
3. A Negative result does not exclude active HCV infection.
4. Performance has not been determined for testing of individuals:
   - without antibody evidence of infection with HCV
   - monitoring HCV-infected patients for progress of disease or response to treatment.
5. It is not known if performance is affected by:
   - the state of HCV infection (acute or chronic)
   - presence or absence of liver disease.
6. **Not intended for use in screening blood, plasma or tissue donors.** The effectiveness of this test for use in screening blood, plasma or tissue donors has not been established.

Summary and Explanation of the Test

HCV causes the most common chronic parenterally transmitted infection in the United States. It is estimated that approximately 1.8% of Americans and 0.6% of Canadians have been infected with HCV[1]. HCV-associated end-stage liver disease is the most frequent indication for liver transplantation among adults, and the Centers for Disease Control and Prevention (CDC) estimate that 8,000-10,000 deaths per year are due to HCV-related chronic liver disease[2].

Reducing the burden of HCV infection will require implementation of primary prevention and secondary prevention activities[2]. Primary preventive measures are aimed at reducing the risk of contracting HCV infection. These include screening of donors of blood and blood components, tissue organ and semen for HCV infection, prevention of illegal injecting drug use and counseling of drug users on how best to reduce the risk of transmitting the infection to others, and implementation of standard barrier precautions in healthcare professionals. Secondary prevention measures are aimed at reducing the risk for liver and other chronic diseases in HCV-infected persons. These include diagnosing HCV infection, and offering appropriate management.

CDC produced recommendations in 1998 outlining which persons should routinely be tested for HCV infection[2]. These include, but are not limited to, persons with persistently abnormal alanine transaminase (ALT) levels, persons who ever injected illegal drugs, certain prior recipients of blood or blood components or an organ transplant, and persons who were ever on chronic hemodialysis. Persons with recognized exposure risk (such as healthcare or emergency workers in contact with blood, or babies born to HCV-infected women) should also be tested.

Available diagnostic tests either detect antibodies to HCV (anti-HCV) or HCV RNA[3,4,5,6]. Anti-HCV indicates prior exposure to HCV but does not distinguish between cleared and active infection (i.e., the virus is replicating). In a person with anti-HCV, detectable HCV RNA indicates active infection. The results of HCV RNA testing can identify patients with active infection and, together with other biochemical and clinical information, may be used to provide counseling and assess whether treatment is appropriate. Following diagnosis, HCV-infected persons can be counseled about protecting the liver from further harm and reducing risk for transmission to others. They can also be advised regarding assessment of liver function and disease severity and available treatment options.

Principles of the Procedure

The COBAS AMPLICOR HCV Test, v2.0 is based on five major processes: specimen preparation, reverse transcription to generate cDNA[7] from target HCV RNA and HCV Internal Control (HCV IC) RNA, PCR amplification[7] of target cDNAs by using HCV-specific primers, hybridization of amplified cDNAs to target-specific oligonucleotide probes, and colorimetric detection of the probe-bound amplified cDNAs.

The COBAS AMPLICOR HCV Test, v2.0 permits simultaneous reverse transcription and PCR amplification of HCV and HCV IC target RNAs. The Master Mix reagent contains a primer pair that is specific for both HCV and HCV IC RNAs. Detection of amplified DNA is performed using target-specific oligonucleotide probes that permit independent identification of HCV amplicon and HCV IC amplicon.

Specimen Preparation

HCV RNA is isolated directly from serum or EDTA plasma by lysing virus particles with a chaotropic agent. HCV IC RNA, introduced into each specimen with Lysis Reagent, serves as an extraction and amplification control for each processed specimen. HCV and HCV IC RNAs are precipitated by using alcohol and then resuspended in Specimen Diluent.

Reverse Transcription and PCR Amplification

Target Selection

Appropriate selection of primers and probe was critical for COBAS AMPLICOR HCV Test, v2.0 detection of all recognized genotypes (see *"Non-Clinical Performance"* section). Accordingly, selection of the target RNA sequence was based on identifying a region of the HCV genome that was maximally conserved among genotypes [8,9,10]. HCV RNA sequences are most conserved in the 5' untranslated region (UTR)[11,12]. The COBAS AMPLICOR HCV Test, v2.0 uses primers KY78 and KY80 to amplify a 5' UTR sequence of 244 nucleotides[13]. HCV RNA sequence corresponding to these primers and the capture probe are located in the most conserved 5' UTR domains[11].

Reverse Transcription

Reverse transcription and amplification reactions are performed with the thermostable recombinant enzyme *Thermus thermophilus* DNA Polymerase (r*Tth* pol). In the presence of manganese (Mn^{2+}) and the appropriate buffer, r*Tth* pol has reverse transcriptase and DNA polymerase activities[7]. This allows both reverse transcription and PCR amplification to occur in the same reaction mixture.

Processed specimens are added to the amplification mixture in amplification tubes (A-tubes) in which reverse transcription and PCR amplification occur. The downstream or antisense primer (KY78) is biotinylated at the 5' end; the upstream or sense primer (KY80) is not biotinylated. The reaction mixture is heated in the COBAS AMPLICOR Analyzer to allow specific annealing of the downstream primer to target HCV and HCV IC RNAs. In the presence of Mn^{2+} and excess deoxynucleoside triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine (in place of thymidine) triphosphates, r*Tth* pol extends the annealed primer to form cDNA.

Target Amplification

Following reverse transcription of target HCV and HCV IC RNAs, the reaction mixture is heated to denature RNA:cDNA hybrids and expose sequences that anneal with the primers. As the mixture cools, the upstream primer (KY80) anneals specifically to the cDNA strand representing each target RNA, r*Tth* pol extends the primer and a second DNA strand is synthesized. This completes the first cycle of PCR, yielding a double-stranded DNA copy of each RNA target region (HCV and HCV IC).

The reaction mixture is heated again to separate the double-stranded DNA and expose the primer-annealing sequences. As the mixture cools, primers KY78 and KY80 anneal to target DNA. The r*Tth* pol enzyme, in the presence of Mn^{2+} and excess dNTPs, extends the annealed primers along the target templates to produce a 244-base pair double-stranded DNA "amplicon". The COBAS AMPLICOR Analyzer automatically repeats this process for 36 cycles, with each cycle intended to double the amount of amplicon DNA. The required number of cycles is preprogrammed into the COBAS AMPLICOR Analyzer. Amplification occurs only in the region of the HCV genome between the primers; the entire genome is not amplified.

HCV Internal Control (HCV IC) Amplification

In enzyme-based amplification processes such as PCR, inhibitors that may be present in the clinical specimen can reduce amplification efficiency. The HCV IC has been added to the COBAS AMPLICOR HCV Test, v2.0 to permit identification of processed specimens containing substances that may interfere with PCR amplification or specimens in which HCV RNA may have been lost during specimen processing. The HCV IC is an RNA transcript with primer-annealing regions identical to those in the HCV genome, a randomized internal sequence of similar length and base composition as the HCV target sequence, and a unique probe-binding region that differentiates HCV IC amplicon from HCV amplicon. These features were selected to ensure equivalent amplification of HCV IC and HCV target RNAs. The HCV IC is introduced into each specimen with the Lysis Reagent and serves as an extraction and amplification control for each processed specimen.

Selective Amplification

Selective amplification of target nucleic acid from the clinical specimen is achieved in the COBAS AMPLICOR HCV Test, v2.0 by the use of AmpErase® (uracil-N-glycosylase) and deoxyuridine triphosphate (dUTP). AmpErase recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[14], but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon from this assay due to the use of deoxyuridine triphosphate in place of thymidine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contain deoxyuridine.

Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase prior to amplification of the target DNA. AmpErase, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step at the alkaline pH of Master Mix, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. AmpErase is inactive at temperatures above 55°C; i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. Following amplification, any residual AmpErase is

denatured by the addition of Denaturation Solution, thereby preventing the degradation of any target amplicon. AmpErase in the COBAS AMPLICOR HCV Test, v2.0 has been demonstrated to inactivate at least 10^3 copies of deoxyuridine-containing HCV amplicon per PCR.

Hybridization Reaction

Following PCR amplification, the COBAS AMPLICOR Analyzer automatically adds Denaturation Solution to the A-tubes to chemically denature the HCV amplicon and the HCV IC amplicon to form single-stranded DNA. Aliquots of denatured amplicon are then transferred to detection cups (D-cups). A suspension of magnetic particles coated with an oligonucleotide probe specific for HCV (KY150) or HCV IC (SK535) is added to the individual D-cups. The biotin-labeled HCV and HCV IC amplicon are hybridized to the target-specific oligonucleotide probes bound to the magnetic particles. This hybridization of amplicon to the target-specific probe increases the overall specificity of the test.

Detection Reaction

Following the hybridization reaction, the COBAS AMPLICOR Analyzer washes the magnetic particles in the D-cup to remove unbound material and then adds Avidin-Horseradish Peroxidase Conjugate. The Avidin-Horseradish Peroxidase Conjugate binds to the biotin-labeled amplicon that was hybridized to target-specific oligonucleotide probe (HCV or HCV IC) bound to the magnetic particles. The COBAS AMPLICOR Analyzer removes unbound conjugate by washing the magnetic particles and then adds a substrate solution containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) to each D-cup. In the presence of hydrogen peroxide, particle-bound horseradish peroxidase catalyzes the oxidation of TMB to form a colored complex, the absorbance of which is measured by the COBAS AMPLICOR Analyzer at 660 nm (A_{660}).

Reagents

AMPLICOR HCV Specimen Preparation Kit, version 2.0 — HCV PREP — 96 Tests — P/N: 21111086 ART: 11 1108 6 US: 83126

HCV LYS, v2.0 — 8 x 6.9 mL

(HCV Lysis Reagent, version 2.0)

- Tris-HCl buffer
- 68% Guanidine thiocyanate
- 3% Dithiothreitol
- < 1% Glycogen

Xn  68% (w/w) Guanidine thiocyanate

Harmful

HCV DIL, v2.0 — 8 x 4.8 mL

(HCV Specimen Diluent, version 2.0)

- Tris-HCl buffer
- < 0.005% Poly rA RNA (synthetic)
- EDTA
- 0.05% Sodium azide

HCV IC, v2.0 8 x 0.1 mL
(HCV Internal Control, version 2.0)
< 0.001% Non-infectious *in vitro* transcribed RNA (microbial) containing HCV primer binding sequences and a unique probe binding region
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

AMPLICOR HCV Controls Kit, version 2.0 HCV CTL 8 Sets P/N: 21111175 ART: 11 1117 5 US: 83131

NHP 8 x 0.6 mL
[Negative Plasma (Human)]
Human plasma, non-reactive by US FDA licensed tests for antibody to HIV-1 and HIV-2, antibody to HCV, HIV p24 antigen and HBsAg
0.1% ProClin® 300

HCV (–) C, v2.0 8 x 0.1 mL
[HCV (–) Control, version 2.0]
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

HCV (+) C, v2.0 8 x 0.1 mL
[HCV (+) Control, version 2.0]
< 0.001% Non-infectious *in vitro* transcribed RNA (microbial) containing HCV sequences
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

AMPLICOR HCV Amplification Kit, version 2.0 HCV AMP 96 Tests P/N: 21111094 ART: 11 1109 4 US: 83127

HCV MMX, v2.0 8 x 0.7 mL
(HCV Master Mix, version 2.0)
Bicine buffer
16% DMSO
Glycerol
< 0.01% r*Tth* DNA Polymerase (r*Tth* pol, microbial)
Potassium acetate
< 0.001% dATP, dCTP, dGTP, dUTP
< 0.005% KY78 and KY80 primers (one is biotinylated)
< 0.01% AmpErase (microbial)
0.05% Sodium azide

HCV Mn^{2+}, v2.0 8 x 0.1 mL
(HCV Manganese Solution, version 2.0)
< 2% Manganese
Acetic acid
Amaranth dye
0.05% Sodium azide

COBAS AMPLICOR HCV Detection Kit, version 2.0 HCV DK 100 Tests P/N: 21111132 ART: 11 1113 2 US: 83130

CX PS1, v2.0 1 x 100 Tests
(HCV Probe Suspension 1, version 2.0)
MES buffer
< 0.4% Suspension of Dynabeads® (paramagnetic particles) coated with HCV-specific oligonucleotide capture probe (KY150)
0.09% Sodium azide

CX4, v2.0 1 x 100 Tests
(HCV Probe Suspension 2, version 2.0)
Sodium phosphate buffer
0.2% Solubilizer
42.2% Sodium thiocyanate

Xn  42.2% (w/w) Sodium thiocyanate
Harmful

COBAS AMPLICOR Internal Control Detection Kit IC DK 100 Tests P/N: 20757608 ART: 07 5760 8 US: 83281

IC PS1 1 x 100 Tests
(IC Probe Suspension 1)
MES buffer
< 0.35% Suspension of Dynabeads® (paramagnetic particles) coated with Internal Control-specific oligonucleotide capture probe (SK535)
0.09% Sodium azide

IC4 1 x 100 Tests
(IC Probe Suspension 2)
Sodium phosphate buffer
0.2% Solubilizer
< 25% Sodium thiocyanate

COBAS AMPLICOR Detection Reagents Kit DK 100 Tests P/N: 20757470 ART: 07 5747 0 US: 83276

DN4 1 x 100 Tests
(Denaturation Solution)
1.6% Sodium hydroxide
EDTA
Thymol blue

Xi  1.6% (w/w) Sodium hydroxide
Irritant

CN4 1 x 100 Tests
(Avidin-Horseradish Peroxidase Conjugate)
Tris-HCl buffer
< 0.001% Avidin-horseradish peroxidase conjugate
Bovine serum albumin (mammalian)
Emulsit 25 (Dai-ichi Kogyo Seiyaku Co., Ltd.)
0.1% Phenol
1% ProClin 150

SB3 5 x 75 Tests
(Substrate A)
Citrate solution
0.01% Hydrogen peroxide
0.1% ProClin 150

SB 5 x 5 mL
(Substrate B)
0.1% 3,3',5,5'-tetramethylbenzidine (TMB)
40% Dimethylformamide (DMF)

T  40% (w/w) Dimethylformamide (DMF)
Toxic

R: 61-20/21-36: May cause harm to the unborn child. Harmful by inhalation and in contact with skin. Irritating to eyes.

S: 53-45: Avoid exposure - obtain special instructions before use. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

COBAS AMPLICOR CN4 200 Tests P/N: 20764213
Conjugate Detection Reagent ART: 07 6421 3
US: 83305

CN4 2 x 100 Tests
(Avidin-Horseradish Peroxidase Conjugate)
Tris-HCl buffer
< 0.001% Avidin-horseradish peroxidase conjugate
Bovine serum albumin (mammalian)
Emulsit 25 (Dai-ichi Kogyo Seiyaku Co., Ltd.)
0.1% Phenol
1% ProClin 150

COBAS AMPLICOR WB 500 Tests P/N: 20759899
Wash Buffer ART: 07 5989 9
US: 83314

WB 2 x 250 Tests
(10X-Wash Concentrate)
< 2% Phosphate buffer
< 9% Sodium chloride
EDTA
< 2% Detergent
0.5% ProClin 300

Warnings and Precautions

For *in vitro* diagnostic use.

This test is only for use with human serum or plasma collected in EDTA (EDTA plasma). ***Heparin has been shown to inhibit PCR and must not be used with this procedure.*** If this test is conducted for a patient who will be receive heparin, (e.g., therapeutically or during hemodialysis), the serum or EDTA plasma specimen should be drawn prior to any administration of heparin.

Do not dilute Potentially Inhibitory specimens prior to retesting. Instead, another aliquot of the original specimen should be extracted and repeat tested. If the original specimen is not available, a new specimen must be collected and tested.

Do not pipet by mouth.

Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.

Avoid microbial and ribonuclease (RNase) contamination of reagents when removing aliquots from reagent bottles. The use of sterile disposable pipets and RNase-free pipet tips is recommended.

Do not pool reagents from different lots or from different bottles of the same lot.

Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

Do not use a kit after its expiration date.

Material Safety Data Sheets (MSDS) are available on request from your local Roche office.

Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Pre-Amplification Area and moving to the Post-Amplification (Amplification/Detection) Area. Pre-amplification activities must begin with reagent preparation and proceed to specimen preparation. Supplies and equipment must be dedicated to each pre-amplification activity and not used for other activities or moved between areas. Disposable gloves must be worn in each area and must be changed before leaving that area. Equipment and supplies used for reagent preparation must not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target DNA. Post-amplification supplies and equipment must remain in the Post-Amplification Area at all times. AmpErase in the COBAS AMPLICOR HCV Test, v2.0 is not intended to substitute for uni-directional workflow.

Specimens should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[15] and in the NCCLS Document M29-A[16]. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

Note

Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.

CAUTION: This kit contains a component (**NHP**) derived from human blood. The source material was non-reactive by US FDA-licensed tests for antibodies to human immunodeficiency virus type 1 (HIV-1) and HIV-2, anti-HCV, HIV p24 antigen and Hepatitis B Surface Antigen (HBsAg). No known test methods can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore all human sourced material should be considered potentially infectious. **NHP** should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[15] and in the NCCLS Document M29-A[16]. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

HCV IC, v2.0; HCV DIL, v2.0; HCV MMX, v2.0; HCV Mn^{2+}, v2.0; HCV (–) C, v2.0; HCV (+) C, v2.0; CX PS1, v2.0 and **IC PS1** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buildup.

Wear eye protection, laboratory coats and disposable gloves when handling **HCV LYS, v2.0; HCV MMX, v2.0; HCV Mn^{2+}, v2.0; CX4, v2.0; IC4; DN4; CN4; SB3; SB** and Working Substrate (mixed **SB3** and **SB** reagent). Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.

Avoid contact between the skin or mucous membranes and **SB** or Working Substrate. If skin contact occurs, wash immediately with large amounts of water.

SB and Working Substrate contain dimethylformamide, which has been reported to be toxic in high oral doses and may be harmful to the unborn child. Skin contact, inhalation of fumes and ingestion must be avoided. If skin contact occurs, wash thoroughly with soap and water and seek medical advice immediately.

Do not allow **HCV LYS, v2.0,** which contains guanidine thiocyanate, or **CX4, v2.0** and **IC4**, which contain sodium thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.

Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens. ***Do not use snap cap tubes.***

Storage and Handling Requirements

Do not freeze reagents.

Store **HCV LYS, v2.0; HCV DIL, v2.0** and **HCV IC, v2.0** at 2-8°C. Unopened, these reagents are stable until the expiration date indicated. Once opened, any unused portion must be discarded.

A precipitate forms in **HCV LYS, v2.0** during storage at 2-8°C. Prior to use, dissolve the precipitate by warming **HCV LYS, v2.0** to 25-37°C. Warm the **HCV LYS, v2.0** for a maximum of 30 minutes followed by mixing thoroughly until the crystals are dissolved. ***Prior to use, examine each bottle of HCV LYS, v2.0 against a white background for appearance of a yellow color or signs of leakage. If there is any yellow color or evidence of leakage, do not use that bottle for testing. Contact your local Roche Office.*** Once opened, any unused portion must be discarded. Working Lysis Reagent (prepared by adding **HCV IC, v2.0** to **HCV LYS, v2.0**) must be stored at room temperature and used within 8 hours of preparation.

Store **HCV MMX, v2.0** and **HCV Mn^{2+}, v2.0** at 2-8°C. These reagents are stable until the expiration date indicated. Once opened, any unused portion must be discarded. Working Master Mix (prepared by addition of **HCV Mn^{2+}, v2.0** to **HCV MMX, v2.0**) must be stored at 2-8°C and used within 4 hours of preparation.

Store **NHP, HCV (–) C, v2.0** and **HCV (+) C, v2.0** at 2-8°C. These reagents are stable until the expiration date indicated. Once opened, any unused portion must be discarded.

Store **CX PS1, v2.0** and **CX4, v2.0** at 2-8°C. These reagents are stable until the expiration date indicated. Once **CX PS1, v2.0** and **CX4, v2.0** are mixed, the Working Reagent is stable for 30 days at 2-8°C. The Working Reagent can be used for a maximum of six instrument cycles (12 hours per cycle) and must be stored at 2-8°C between instrument cycles.

Store **IC PS1** and **IC4** at 2-8°C. These reagents are stable until the expiration date indicated. Once **IC PS1** and **IC4** are mixed, the Working Reagent is stable for 30 days at 2-8°C. The Working Reagent can be used for a maximum of six instrument cycles (12 hours per cycle) and must be stored at 2-8°C between instrument cycles.

Store **DN4** at 2-25°C. **DN4** is stable until the expiration date indicated. Once opened, **DN4** is stable for 30 days at 2-8°C or until the expiration date, whichever comes first. **DN4** can be used for a maximum of six instrument cycles (12 hours per cycle) and must be stored at 2-8°C between instrument cycles.

Store **CN4** at 2-8°C. **CN4** is stable until the expiration date indicated. Once opened, this reagent is stable for 30 days at 2-8°C or until the expiration date, whichever comes first. **CN4** can be used for a maximum of six instrument cycles (12 hours per cycle) and must be stored at 2-8°C between instrument cycles.

Store **SB3** and **SB** at 2-8°C. Unopened, these reagents are stable until the expiration dates indicated. Working Substrate must be prepared each day by mixing **SB3** with **SB**. Working Substrate is stable on the COBAS AMPLICOR Analyzer for 16 hours. Do not expose **SB3, SB** or Working Substrate to metals, oxidizing agents or direct light.

Store **WB** at 2-25°C. **WB** is stable unopened until the expiration date indicated. **WB** is stable after opening for at least 5 months. Examine **WB** before dilution, and if necessary, warm at 30-37°C to redissolve any precipitate. Working Wash Buffer (1X), prepared by diluting **WB** 1:10 with distilled or deionized water, should be stored at 2-25°C in the COBAS AMPLICOR Wash Buffer Reservoir and is stable for 2 weeks from the date of preparation.

Store partially used detection reagents at 2-8°C between instrument runs. Check expiration date of opened or Working Reagents prior to loading on the COBAS AMPLICOR Analyzer.

Materials Provided

AMPLICOR HCV
Specimen Preparation Kit, version 2.0
HCV PREP
P/N: 21111086
ART: 11 1108 6
US: 83126

HCV LYS, v2.0
(HCV Lysis Reagent, version 2.0)
HCV DIL, v2.0
(HCV Specimen Diluent, version 2.0)
HCV IC, v2.0
(HCV Internal Control, version 2.0)

AMPLICOR HCV
Controls Kit, version 2.0
HCV CTL
P/N: 21111175
ART: 11 1117 5
US: 83131

NHP
[Negative Plasma (Human)]
HCV (–) C, v2.0
[HCV (–) Control, version 2.0]
HCV (+) C, v2.0
[HCV (+) Control, version 2.0]

AMPLICOR HCV
Amplification Kit, version 2.0
HCV AMP
P/N: 21111094
ART: 11 1109 4
US: 83127

HCV MMX, v2.0
(HCV Master Mix, version 2.0)
HCV Mn^{2+}, v2.0
(HCV Manganese Solution, version 2.0)

COBAS AMPLICOR HCV
Detection Kit, version 2.0
HCV DK
P/N: 21111132
ART: 11 1113 2
US: 83130

CX PS1, v2.0
(HCV Probe Suspension 1, v2.0)
CX4, v2.0
(HCV Probe Suspension 2, v2.0)

COBAS AMPLICOR
Internal Control Detection Kit
IC DK
P/N: 20757608
ART: 07 5760 8
US: 83281

IC PS1
(IC Probe Suspension 1)
IC4
(IC Probe Suspension 2)

COBAS AMPLICOR
Detection Reagents Kit

DK

P/N: 20757470
ART: 07 5747 0
US: 83276

DN4
(Denaturation Solution)
CN4
(Avidin-Horseradish Peroxidase Conjugate)
SB3
(Substrate A)
SB
(Substrate B)

COBAS AMPLICOR
Conjugate Detection Reagent

CN4

P/N: 20764213
ART: 07 6421 3
US: 83305

CN4
(Avidin-Horseradish Peroxidase Conjugate)

COBAS AMPLICOR
Wash Buffer

WB

P/N: 20759899
ART: 07 5989 9
US: 83314

WB
(10X-Wash Concentrate)

Materials Required but Not Provided

Pre-Amplification – Reagent Preparation Area

- COBAS AMPLICOR A-ring fitted with 12 A-tubes (ART: 10 4563 6)
- COBAS AMPLICOR A-ring holder
- Plastic resealable bag
- Eppendorf Repeater® pipet with 1.25 mL Combitip® Reservoir (sterile, individually wrapped).
- Pipettors (capacity 50 µL and 100 µL)* with aerosol barrier or positive displacement RNase-free tips
- Disposable gloves, powderless

Pre-Amplification – Specimen and Control Preparation Area

- 1.5 mL polypropylene screw-cap tubes, sterile, non-siliconized, conical (Sarstedt 72.692.005 or equivalent)**
- Tube racks (Sarstedt 93.1428 or equivalent)
- 95% ethanol, reagent grade for Microbiology or Histology use (freshly diluted to 70% using distilled or deionized water)
- Isopropyl alcohol, reagent grade
- Sterile fine-tip transfer pipets, RNase-free
- Sterile disposable, polystyrene serological pipets (5 mL, 10 mL and 25 mL)
- Pipettors (capacity 20 µL, 50 µL, 100 µL, 200 µL, 400 µL, 600 µL and 1000 µL)* with aerosol barrier or positive displacement RNase-free tips
- Microcentrifuge (max. RCF 16,000 x g, min. RCF 12,500 x g); Eppendorf 5415C, HERMLE Z230M, or equivalent
- Vortex mixer
- 60°C ± 2°C dry heat block
- Disposable gloves, powderless

Post-Amplification – Amplification/Detection Area

- COBAS AMPLICOR Analyzer and printer
- *Operator's Manual* for the COBAS AMPLICOR Analyzer
- *Method Manual* for the COBAS AMPLICOR Hepatitis C Virus Test, v2.0
- Racks of D-cups (ART: 10 4564 4)
- Distilled or deionized water
- 5 mL serological pipets
- Disposable gloves, powderless

* Pipettors should be accurate within 3% of stated volume. Aerosol barrier or positive displacement RNase-free tips must be used where specified to prevent specimen and amplicon cross-contamination.

** Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens and controls. ***Do not use snap cap tubes.***

Specimen Collection, Transport and Storage

Note ***Handle all specimens as if they are capable of transmitting infectious agents.***

Specimen Collection The COBAS AMPLICOR Hepatitis C Virus Test, v2.0 is for use with serum or EDTA plasma specimens only. Blood should be collected in SST® Serum Separation Tubes, in sterile collection tubes with no additives (red tops), or in sterile tubes using EDTA (lavender top). ***Specimens collected using heparin as the anticoagulant are unsuitable for this test.*** Store whole blood at 2-25°C for no longer than 6 hours.

Separate serum or EDTA plasma from whole blood within 6 hours of collection by centrifugation at 1500 x g for 20 minutes at room temperature. Transfer serum or EDTA plasma to a sterile, screw-cap polypropylene tube.

Specimen Transport Transportation of whole blood, serum or EDTA plasma must comply with country, federal, state and local regulations for the transport of etiologic agents[17]. Whole blood must be transported at 2-25°C and processed within 6 hours of collection. Serum or EDTA plasma may be transported at 2-8°C or frozen at -70° or colder, and must be processed within the limits specified below.

Specimen Storage Serum or EDTA plasma specimens may be stored at 2-8°C for up to 72 hours or frozen at -70°C or colder indefinitely. It is recommended that specimens be stored in 250-300 µL aliquots in sterile, 1.5 mL polypropylene screw-cap tubes (such as Sarstedt 72.692.005). Serum or EDTA plasma specimens may be frozen and thawed up to three times without a loss of HCV RNA.

Instructions for Use

Note ***For detailed operating instructions, refer to the Operator's Manual for the COBAS AMPLICOR Analyzer.***

Note ***All reagents must be at room temperature before use. Visually examine reagents for sufficient reagent volume before beginning the test procedure.***

Note ***Serum and plasma specimens must be at room temperature before use.***

Note ***Use pipettors with aerosol barrier or positive displacement tips where specified. Use extreme care to ensure selective amplification.***

Note ***Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens and controls. Do not use snap cap tubes.***

Run Size Each kit contains reagents sufficient for eight 12-test runs, which may be performed separately or simultaneously. At least one replicate each of the AMPLICOR HCV (–) Control and the AMPLICOR HCV (+) Control must be included in each test run (see *"Quality Control"* section).

The Specimen Preparation and Amplification Reagents are packaged in 12-test, single-use bottles. The HCV (–) and HCV (+) Controls are packaged in single-use vials. For the most efficient use of reagents, specimens and controls should be processed in batches that are multiples of 12.

Workflow

The COBAS AMPLICOR Hepatitis C Virus Test, v2.0 can be completed in one day or over two days. If the testing is to be completed in a single work day, follow the instructions in *Reagent Preparation, Specimen and Control Preparation, and Reverse Transcription, Amplification and Detection* in order. Testing can be completed over 2 days by starting *Specimen and Control Preparation* on Day 1, followed by *Reagent Preparation and Reverse Transcription, Amplification and Detection* on Day 2 or by starting *Specimen and Control Preparation,* and *Reverse Transcription and Amplification* on Day 1 and *Detection* on Day 2.

To perform specimen and control processing on Day 1, and reverse transcription, amplification and detection on Day 2, perform *Specimen and Control Preparation* (Steps 1 through 15) and store the processed specimens and controls as indicated in Step 15. On Day 2, begin with *Reagent Preparation,* then thaw the processed specimens and controls at room temperature, and continue with *Specimen and Control Preparation,* Step 16, through *Reverse Transcription, Amplification and Detection.*

To perform specimen and control processing and reverse transcription and amplification on Day 1, proceed with all steps of *Reagent Preparation* and *Specimen and Control Preparation.* Program the COBAS AMPLICOR Analyzer in the Parallel mode and complete the amplification steps (See the *Operator's Manual* for the COBAS AMPLICOR Analyzer for detailed instructions). Store the denatured amplicon overnight at 2-8°C and continue with Detection on Day 2.

Reagent Preparation

Performed in: Pre-Amplification – Reagent Preparation Area

1. Determine the appropriate number of A-ring(s) needed for patient specimen and control testing. Place the A-ring(s) on the A-ring holder(s).

2. Prepare Working Master Mix by adding 100 µL **HCV Mn^{2+}, v2.0** to one vial **HCV MMX, v2.0**. *It is not necessary to measure the volume of Master Mix. Add 100 µL of HCV Mn^{2+}, v2.0 to the entire vial of HCV MMX v2.0.* Recap the tube and mix well by inverting the tube 10-15 times. Do not vortex the Working Master Mix. The pink dye in **HCV Mn^{2+}, v2.0** is used for visual confirmation that **HCV Mn^{2+}, v2.0** has been added to **HCV MMX, v2.0**. Discard remaining **HCV Mn^{2+}, v2.0**. Working Master Mix must be stored at 2-8°C and used within 4 hours of preparation.

3. Add 50 µL of Working Master Mix into each A-tube using a repeat pipettor or a pipettor with an aerosol barrier or positive displacement tip. Do not close the covers of the A-tube(s) at this time.

4. Place the A-ring(s) containing Working Master Mix in a resealable plastic bag and seal the bag securely. Move the A-ring(s) to the Pre-Amplification – Specimen and Control Preparation Area. Store the A-ring(s) containing Working Master Mix at 2-8°C in the Pre-Amplification – Specimen and Control Preparation Area until specimen and control preparation is completed. Working Master Mix is stable for 4 hours at 2-8°C in A-tubes sealed in the plastic bag.

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Specimen and Control Preparation

Performed in: Pre-Amplification – Specimen and Control Preparation Area

Note ***To amplify previously processed specimens and controls, first perform the steps in the "Reagent Preparation" section. Thaw processed specimens and controls at room temperature and continue with "Specimen and Control Preparation", Step 16.***

Note ***Prior to use, examine each bottle of HCV LYS, v2.0 against a white background for the appearance of a yellow color or signs of leakage. If there is any yellow color or evidence of leakage, do not use that bottle for testing. Contact your local Roche Office.***

Note ***A precipitate forms in HCV LYS, v2.0 upon storage at 2-8°C. Prior to use, warm at 25-37°C for a maximum of 30 minutes and mix thoroughly to dissolve the precipitated material.***

1. Prepare 70% ethanol. For 12 tests, mix 11.0 mL 95% ethanol and 4.0 mL of deionized or distilled water.

2. Label one 1.5 mL screw-cap tube for each patient specimen and label two additional tubes as "HCV (–) C" and "HCV (+) C". There is no specific requirement regarding the position of the controls in the run.

3. Prepare Working Lysis Reagent. Vortex **HCV IC, v2.0** for 5-10 seconds before use. For each batch of up to 12 specimens and controls, add 100 µL **HCV IC, v2.0** to one bottle **HCV LYS, v2.0** and mix well. It is not necessary to measure the volume of **HCV LYS, v2.0**.

Note ***Discard the remaining HCV IC, v2.0. Working Lysis Reagent is stable for 8 hours at room temperature.***

Note ***If using frozen specimens, thaw the specimens at room temperature and vortex for 3 - 5 seconds. Spin the specimen tube briefly to collect specimen in base of tube. Take care to avoid contaminating gloves when manipulating specimens.***

4. Add 400 µL of Working Lysis Reagent to each of the labeled tubes and cap the tubes.

5. Prepare Controls as follows:

   - Vortex **NHP, HCV (–) C, v2.0** and **HCV (+) C, v2.0** for 5-10 seconds.

   - Add 200 µL **NHP** to each of the two control tubes. Cap the tubes and vortex for 3-5 seconds.

   - Add 20 µL **HCV (–) C, v2.0** to the tube labeled "HCV (–) C" containing Working Lysis Reagent and **NHP**. Cap the tube and vortex for 3-5 seconds.

   - Add 20 µL **HCV (+) C, v2.0** to the tube labeled "HCV (+) C" containing Working Lysis Reagent and **NHP**. Cap the tube and vortex for 3-5 seconds.

6. Add 200 µL of each patient specimen to the appropriately labeled tube containing Working Lysis Reagent. Cap the tubes and vortex for 3-5 seconds.

7. Incubate the specimen and control tubes in a dry heat block for 10 minutes at 60°C ± 2°C. Vortex for at least 10 seconds.

8. Remove the caps from the tubes and add 600 µL 100% isopropyl alcohol (at room temperature) to each tube. Recap the tubes and vortex for 3-5 seconds. Incubate all tubes for 2 minutes at room temperature.

9. Put an orientation mark on each tube and place the tubes in the microcentrifuge with the orientation mark facing outward, so that the pellet will align with the orientation mark. Centrifuge specimens and controls at 12,500-16,000 x g for 15 minutes at room temperature.

10. Using a new, fine-tip disposable transfer pipet for each tube, carefully remove and discard the supernatant from each tube, being careful not to disrupt the pellet (which may not be visible). Remove as much liquid as possible without disturbing the pellet. Withdraw the supernatant slowly, allowing the liquid to drain completely off the sides of the tube. Do not use vacuum aspiration.

11. Add 1.0 mL 70% ethanol (at room temperature) to each tube, recap and vortex for 3-5 seconds.

12. Place the tubes into a microcentrifuge with the orientation marks facing outward and centrifuge the tubes for 5 minutes at 12,500-16,000 x g at room temperature.

13. Using a new, fine-tip disposable transfer pipet for each tube, carefully remove and discard the supernatant without disturbing the pellet. The pellet should be clearly visible at this step. Remove as much of the supernatant as possible.

14. Recap the tubes and centrifuge at maximum speed for 3-5 seconds. Carefully remove the supernatant without disturbing the pellet using a 200 µL capacity pipettor fitted with a new tip for each tube. The pellet should be clearly visible at this step. Remove as much of the supernatant as possible. ***Residual ethanol can inhibit the amplification.***

15. Add 200 µL **HCV DIL, v2.0** to each tube. Break apart the pellet as much as possible with a 200 µL capacity pipettor fitted with an aerosol barrier tip. Recap the tubes. Vortex vigorously for 10 seconds. Some insoluble material may remain. Amplify the processed specimens and controls within 3 hours of preparation or store frozen at -70°C or colder for up to one month with no more than two freeze-thaws. More than two freeze-thaw cycles may result in loss of HCV or HCV IC signal.

Note ***If processed specimens and controls were stored frozen prior to amplification, thaw at room temperature and vortex for 5 seconds before proceeding to Step 16.***

16. Add 50 µL of each processed patient specimen and control to appropriate A-tubes containing Working Master Mix using a micropipettor with an aerosol barrier or positive displacement tip. Use a new tip for each specimen and control. Be careful to avoid transferring any precipitated material that may not have gone back into solution. Cap the A-tubes.

17. Record the positions of the controls and specimens in the A-rings. *Reverse Transcription and Amplification must be started within 45 minutes (or sooner) from the time that the processed specimens and controls are added to the A-tubes containing Working Master Mix.* Move the processed specimens and controls in the A-rings to the Amplification/Detection Area. Start the amplification as soon as possible, but no later than 45 minutes after the processed specimens and controls are added to the A-rings containing Working Master Mix, to ensure optimal performance of the assay. The remainder of the processed specimen may be frozen at -70°C or colder for up to one month. Processed specimens can be frozen and thawed no more than two times. More than two freeze-thaws may result in loss of HCV RNA.

Reverse Transcription, Amplification and Detection

Performed in: Post Amplification – Amplification/Detection Area

Perform Daily Instrument Maintenance as outlined in the *Operator's Manual* for the COBAS AMPLICOR Analyzer including:

- Wipe initialization post with a lint-free moist cloth and dry
- Wipe D-cup handler tip with a lint-free moist cloth and dry
- Check Wash Buffer Reservoir and fill if necessary
- Prepare Working Wash Buffer (1X) as follows: Examine **WB** (10X-Wash Concentrate), and if necessary, warm to 30-37°C to redissolve any precipitate. Add 1 volume of **WB** to 9 volumes of distilled or deionized water. Mix well. Keep a minimum of 3-4 liters of Wash Buffer (1X) in the Wash Buffer Reservoir of the system at all times.
- Empty waste container
- Prime the system
- During the priming, check syringes and tubing
- During the priming, check transfer tip

Prior to each run

- Check waste container and empty if necessary
- Check Wash Buffer Reservoir and add buffer if necessary
- Replace used D-cup racks
- Prime the system

Instrument Loading and System Operation

1. Examine the quantities of reagents on board the COBAS AMPLICOR Analyzer. Prepare enough reagent cassettes to complete the workload.

2. Mix **CX PS1, v2.0** well by vortexing. Add 2.5 mL **CX PS1, v2.0** to one **CX4, v2.0** cassette. Place the cassette on the test specific reagent rack. Discard the used **CX PS1, v2.0** vial. Record the date of reagent preparation on **CX4, v2.0** cassette.

3. Mix **IC PS1** well by vortexing. Add 2.5 mL **IC PS1** to one **IC4** cassette. Place the cassette on the test specific reagent rack. Discard the used **IC PS1** vial. Record the date of reagent preparation on **IC4** cassette.

4. Prepare the Working Substrate by pipetting 5 mL **SB** into one **SB3** cassette. Pipet up and down to mix. Discard the empty **SB** vial. Record the date of preparation on **SB3** cassette.

5. Place the Working Substrate in the generic reagent rack.

6. Place **DN4** and **CN4** cassettes in the generic reagent rack. Record the date each cassette was opened on the cassette.

7. Identify the reagent racks as generic or test specific using the keypad or bar-code scanner as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer or using the AMPLILINK™ software as described in the *Operator's Manual* for the AMPLILINK software.

8. Configure the reagent racks by inputting reagent positions and lot numbers into the Analyzer using the keypad or barcode scanner as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer or using the AMPLILINK software as described in the *Operator's Manual* for the AMPLILINK software.

9. Load the reagent racks onto the Analyzer using the keypad or barcode scanner as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer or using the AMPLILINK software as described in the *Operator's Manual* for the AMPLILINK software. Make sure that each reagent cassette is in its assigned position and that each cassette fits tightly into its rack.

10. Place the D-cup rack on the D-cup platform. One D-cup is required for each detection reaction and two D-cups are required for each cassette of Working Substrate to allow for blanking by the COBAS AMPLICOR Analyzer.

11. Place the A-ring(s) into the thermal cycler segment(s) of the COBAS AMPLICOR Analyzer.

12. Load the A-ring(s) into the Analyzer using the keypad or barcode scanner as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer or using the AMPLILINK software as described in the *Operator's Manual* for the AMPLILINK software.

13. Create an A-ring Worklist as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer.

14. Tightly close the cover of the thermal cycler segment(s).

15. Start the COBAS AMPLICOR Analyzer as described in the *Operator's Manual* for the COBAS AMPLICOR Analyzer.

16. Wait for Analyzer to indicate that the load check has passed.

Note ***The required quantity of each detection reagent is calculated by the Analyzer, and a load check performed at the start of each run determines if sufficient reagents are available for the requested tests.***

17. Reverse transcription, amplification and detection are automatically performed by the COBAS AMPLICOR Analyzer. Results are expressed as absorbance value at 660 nm and as Positive, Negative or Equivocal (COBAS AMPLICOR Analyzer flag "GZ").

Quality Control

At least one replicate of the AMPLICOR HCV (–) Control and one replicate of the AMPLICOR HCV (+) Control must be processed and included with each test run. There are no requirements regarding the position of the controls in the A-ring(s). In addition, the HCV Internal Control (HCV IC) must be added to each specimen and control during Specimen Preparation.

Check run printout for flags and comments to ensure that the run is valid. Refer to the *Operator's Manual* for the COBAS AMPLICOR Analyzer for printing and interpretation of flags and comments.

After addition of HCV IC to specimens and controls, the concentration of HCV IC RNA is ≈400 copies per mL, which corresponds to ≈150 *IU*/mL* of HCV RNA. RNA concentration in the AMPLICOR HCV (+) Control is ≈120 *IU*/mL.

Since the AMPLICOR HCV (+) Control does not control for the lysis portion of Specimen Preparation, the user may consider a well-characterized, HCV RNA-positive specimen that is available in sufficient quantity to be included as an external control for the entire procedure. Additional external controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.

* IU designates International Units of the WHO International Standard for HCV genotype 1 RNA that contains, by definition based on consensus studies, 10^5 IU/mL of genotype 1 HCV RNA[18]. It is available, as NIBSC code 96/790, from the National Institute for Biological Standards and Control, London, U.K. (http://www.nibsc.ac.uk).

In this Method Manual, *IU* designates virion or subgenomic HCV RNA that has been quantified with reference to the WHO Standard. *IU*/mL affords a standardized approach to indicating [HCV RNA] but it is not known if *IU*/mL accurately reflects [HCV RNA] of any particular specimen. Available data indicate that 1 IU corresponds to > 1 HCV RNA molecule and that this number of molecules varies according to quantifying methods (which are imprecise with a single determination) and other variables:

Source of data	Type of RNA	How quantified	Quantifier	Conversion factor
Consensus (ref. 18)	HCV (virion)	End-point dilution: qualitative assays	Copies	1 IU ≈1.8 copies
		Quantitative assays	Copies or genome equivalents	1 IU ≈ 6.6 copies
Roche Molecular Systems (unpublished)	Subgenomic transcript of cloned HCV cDNA	UV spectroscopy	A_{260}-molecules	1 IU ≈ 2.7 A_{260}-molecules (95% CI, 2.6-2.8)

While Roche Molecular Systems has demonstrated similar conversion factors for certain RNAs representing HCV genotypes 2-6, it is not known if quantitation with reference to the WHO Standard is affected by genotype, strain characteristics (including RNA structure and quasispecies), or [HCV RNA]. For example, IU-to-copy ratios for low [HCV RNA] may be different than those for high [HCV RNA].

Negative Control

- The HCV A_{660} absorbance value for the AMPLICOR HCV (–) Control must be < 0.1.
- The HCV IC A_{660} must be ≥ 0.15.
- If HCV A_{660} is ≥ 0.1 or if HCV IC A_{660} is < 0.15 for the AMPLICOR HCV (–) Control, the entire run is invalid. Repeat the entire process (Specimen and Control Preparation, Reverse Transcription, Amplification and Detection).

If the HCV A_{660} for the AMPLICOR HCV (–) Control is consistently ≥ 0.1, contact your local Roche office for technical assistance.

Positive Control

- The HCV A_{660} for the AMPLICOR HCV (+) Control must be ≥ 1.0.
- HCV IC A_{660} must be ≥ 0.15.
- If HCV A_{660} is < 1.0 or if the HCV IC A_{660} is < 0.15 for the AMPLICOR HCV (+) Control, the entire run is invalid. Repeat the entire test (Specimen and Control Preparation, Reverse Transcription, Amplification and Detection).

If the HCV A_{660} for the AMPLICOR HCV (+) Control is consistently < 1.0, contact your local Roche office for technical assistance.

Note

If the HCV IC A_{660} absorbance value for specimens, AMPLICOR HCV (–) Control or AMPLICOR HCV (+) Control is consistently < 0.15, contact your local Roche office for technical assistance.

Results

Interpretation of Results

Note ***Refer to the Operator's Manual for the COBAS AMPLICOR Analyzer for printing results and for the interpretation of flags and comments.***

1. Check run printout for flags (FLG) and comments to ensure that the run is valid. If the run is invalid, repeat the entire run (specimen preparation, amplification and detection).

2. For a valid run, specimen results are interpreted as follows:

HCV A_{660} Result		HCV IC A_{660} Result		Interpretation
A_{660}	COBAS Flag	A_{660}	COBAS Flag	
< 0.15	NEGATIVE	≥ 0.15	POSITIVE	**Negative*:** HCV RNA not detected. This result does not preclude the presence of HCV RNA if specimen handling (collection, transport, processing or storage) was inadequate, interfering substances or inhibitors were present, or RNA was insufficient. (See *"Procedural Limitations"* section for further information).
< 0.15	NEGATIVE	< 0.15	NEGATIVE	**Potentially Inhibited*:** HCV RNA, if present, was not detectable because the specimen contained an inhibitor, or RNA was lost during specimen preparation. Inhibitors are often labile so process another aliquot of specimen and repeat test. If the same result is obtained on repeat testing (i.e. HCV and HCV IC A_{660} both < 0.15), the interpretation remains **Potentially Inhibited.**
≥ 1.0	POSITIVE	ANY	ANY	**Positive*:** HCV RNA detected.
≥ 0.15, < 1.0	GZ 0.15 - 0.99	ANY	ANY	**Equivocal*:** inconclusive for HCV* RNA. Repeat entire test procedure in duplicate, using new aliquots of specimen. When both repeat HCV A_{660} are ≥ 1.0, final interpretation is **Positive** (per above). When both repeat HCV A_{660} are < 0.15 and both HCV IC A_{660} are ≥ 0.15, final interpretation is **Negative** (per above). For any other combination of repeat test results, the interpretation is **Equivocal.**

* Test not verified in (i) the absence of liver disease or antibody evidence of HCV infection or (ii) for monitoring progress of Hepatitis C, including response to treatment (See *"Intended Use"* section).

Procedural Precautions

Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Pre-Amplification Area and moving to the Post-Amplification (Amplification/Detection) Area. Pre-amplification activities must begin with reagent preparation and proceed to specimen preparation. Supplies and equipment must be dedicated to each Pre-amplification activity and not used for other activities or moved between areas. Disposable gloves must be worn in each area and must be changed before leaving that area. Equipment and supplies used for reagent preparation must not be used for specimen preparation activities or for pipetting or processing amplified DNA or other sources of target DNA. Post-amplification supplies and equipment must remain in the Post-Amplification Area at all times. AmpErase in the COBAS AMPLICOR HCV Test, v2.0 is not intended to substitute for uni-directional workflow.

As with any test procedure, good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be visually inspected prior to use. Discard any reagents that may be suspect.

Procedural Limitations

This test has been verified for use with only human serum or plasma from blood collected in EDTA. Testing of other specimen types may result in False Negative (HCV RNA present but $A_{660} < 0.15$) or False Positive (no HCV RNA but $A_{660} \geq 1.0$) results.

Heparin inhibits PCR; specimens collected using heparin as the anticoagulant should not be used with the COBAS AMPLICOR HCV Test, v2.0.

Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.

Although RNA representing all recognized HCV genotypes (1-6) can be detected with this test, analytical sensitivity and other performance characteristics have not been determined for all HCV genotypes.

Detection of HCV RNA is dependent on the number of virions in the specimen and may be affected by specimen collection methods, patient factors and/or state of infection.

False Negative results may occur due to polymerase inhibition or loss of HCV RNA. The HCV IC has been added to the COBAS AMPLICOR HCV Test, v2.0 to permit the identification of processed specimens containing substances which may interfere with PCR amplification or that have lost HCV and HCV IC RNAs. The HCV IC does not control for loss of HCV RNA due to inadequate collection, transport or storage of serum or EDTA plasma specimens.

The effect of cryoglobulins on the COBAS AMPLICOR HCV Test, v2.0 has not been determined. Negative HCV RNA results from specimens known to contain high levels of cryoglobulins should be interpreted with caution.

The effect of elevated concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transferase (GGT) on the COBAS AMPLICOR HCV Test, v2.0 has not been determined.

The effect of therapeutic drugs for bacterial and fungal infections on the COBAS AMPLICOR HCV Test, v2.0 has not been determined.

The presence of AmpErase in the HCV Master Mix reduces the risk of amplicon contamination. However, contamination from HCV-positive controls and clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Method Manual.

As with any diagnostic test, results from the COBAS AMPLICOR HCV Test, v2.0 should be interpreted with consideration of all clinical and laboratory findings.

Use of this product should be limited to personnel who have been trained in the techniques of COBAS AMPLICOR PCR assays.

This product can only be used with the COBAS AMPLICOR Analyzer.

Expected Values

Frequency Distributions of A_{660} Values (Results in Negative, Equivocal and Positive Zones)

Clinical specimens representative of indication for use

A clinical study was performed at four diverse sites in Florida, Georgia, Virginia and Washington, which yielded the data to support the indicated use for the COBAS AMPLICOR HCV Test, v2.0 (see "*Intended Use*" section). A total of 573 specimens (431 frozen sera, 44 refrigerated sera and 98 frozen EDTA plasma) from 478 patients with antibody evidence of HCV infection were tested with the COBAS AMPLICOR HCV Test, v2.0.* While HCV RNA was not characterized via quantification or genotyping, the patients were assumed to represent U.S. populations with active HCV infection and chronic liver disease; i.e., range of HCV RNA concentrations (majority likely > 10^4 *IU*/mL) and HCV genotypes (majority of genotype 1 viruses, with genotypes 2 and 3 comprising most of the remainder).

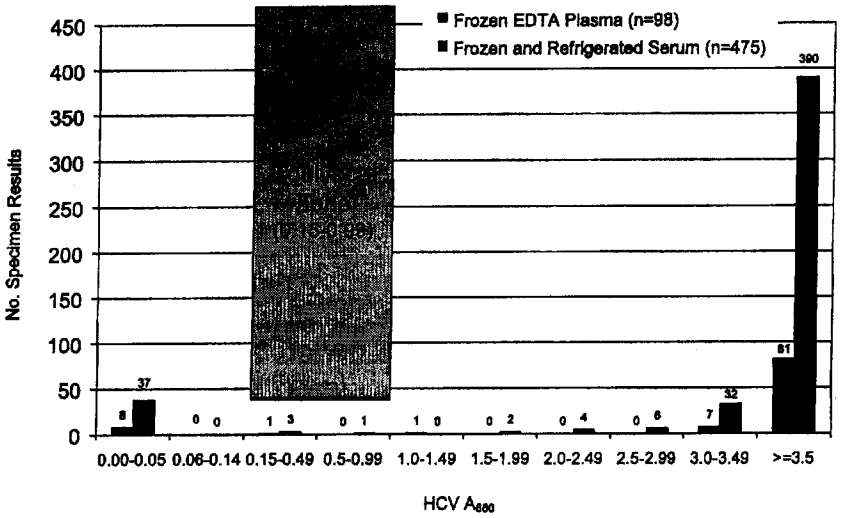
Figure 1 shows distributions of HCV A_{660} values from these specimens, including those from specimens that initially yielded a Potentially Inhibited or Equivocal result (for definitions and repeat testing algorithms, See "*Interpretation of Results*" section). These A_{660} patterns represent those to be expected for appropriate patients. The frequencies of results from serum and EDTA plasma are nearly identical within each HCV A_{660} interval of Figure 1.

Potentially Inhibited (PI) and Equivocal (EZ) results

Among the 573 tested specimens from patients with evidence of anti-HCV, 13 initially yielded results for which repeat testing was appropriate (see "*Interpretation of Results*" section): 8 PI and 5 EZ. The frequencies of these types of results, and results of repeat testing, are provided in Tables 1 and 2.

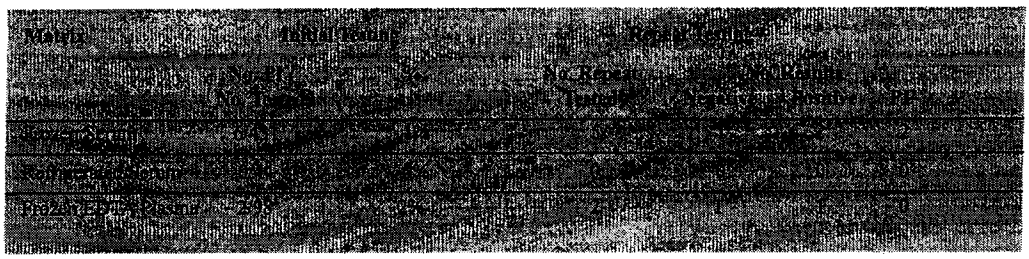
* The study also included patients who were previously treated for HCV and from patients without antibody evidence of HCV infection; their data are described in the "*Clinical Evaluation*" section.

Figure 1
*Distribution of COBAS AMPLICOR HCV Test, v2.0 Values from Untreated Patients with Antibody Evidence of HCV Infection**



* Most HCV A_{660} values are not arithmetically proportional to the HCV RNA concentration in the specimen or that of amplified cDNA from the assay. Specimens were from patients who had repeatedly-reactive results from an anti-HCV enzyme immunoassay (EIA-RR) and evidence of liver disease but had not received antiviral therapy for hepatitis C.

Table 1
Specimens Initially Yielding Potentially Inhibited (PI) Results

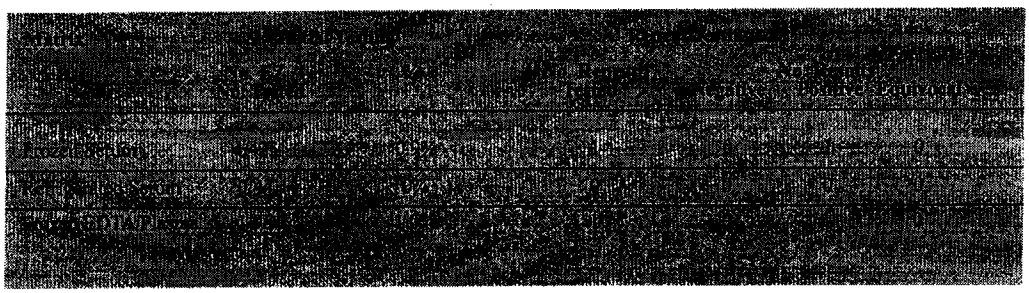


1 Repeat testing and interpretation per *"Interpretation of Results"* section; excludes specimens for which quantity was not sufficient for repeat testing

2 Remained PI after repeat testing

55

Table 2
Specimens Initially Yielding Results in Equivocal Zone (EZ)



[1] Excluded 8 specimens with PI results because the possibility of an Equivocal result was precluded by the PI result.

[2] Repeat testing and interpretation per *"Interpretation of Results"* section; excludes specimens for which quantity was not sufficient for repeat testing.

Equivocal results can be caused by improper technique in running the assay, failure to follow the instructions for specimen handling (resulting in loss of RNA from the specimen) or a specimen with unusually low HCV RNA concentration (generally, < 100 *IU*/mL) may be associated with increased frequencies of Equivocal results. In addition, contamination of an HCV RNA-negative specimen has the potential to produce an Equivocal result. If multiple Equivocal results continue to occur within runs, contact your Roche representative for technical support.

Controls and Run Failures

In the clinical study, there were 200 runs (with 201 sets of controls) of the COBAS AMPLICOR HCV Test, v2.0. The distribution of control values from these runs is provided in Figure 2. During the study, there were 10 runs which failed due to Positive Controls (5%) out of range (i.e., $A_{660} < 1.0$) and 7 runs which failed due to Negative Controls (3.5%) out of range (i.e., $A_{660} \geq 0.1$). In addition, there were 4 failed runs due to protocol deviations (i.e., failure to follow procedures for specimen handling or correct instrument profile setup) and 5 system failures (i.e. COBAS failures or incomplete runs due to improper instrument set-up or mechanical failure). Results of Potentially Inhibited specimens are discussed above in this section. Table 3 provides the frequency of run failures by site, and reasons for failures.

Figure 2
Distribution of COBAS AMPLICOR HCV Test, v2.0 Positive and Negative Control Values for Valid and Invalid A-rings

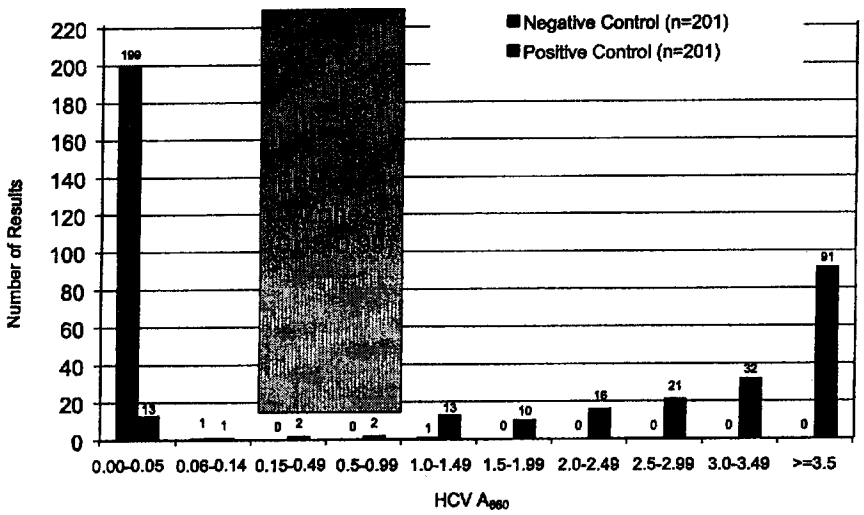
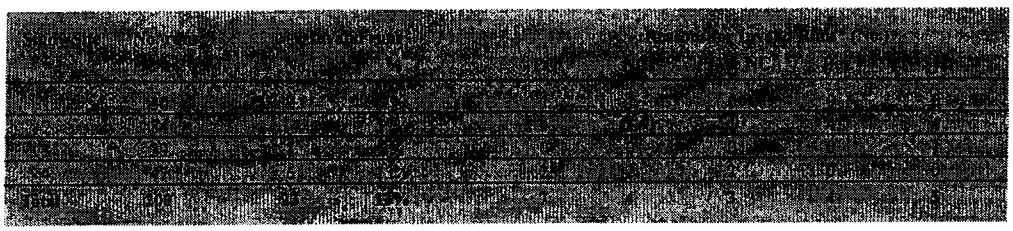


Table 3
Invalid Runs and Reasons for Invalid Runs During the Clinical Study



* PC, Positive control HCV A_{660} < 1.0; NC, Negative control HCV A_{660} ≥ 0.1; Dev, deviations from protocol procedures for specimen handling; Sys, system failures.

Non-Clinical Performance Evaluation

Analytical Sensitivity

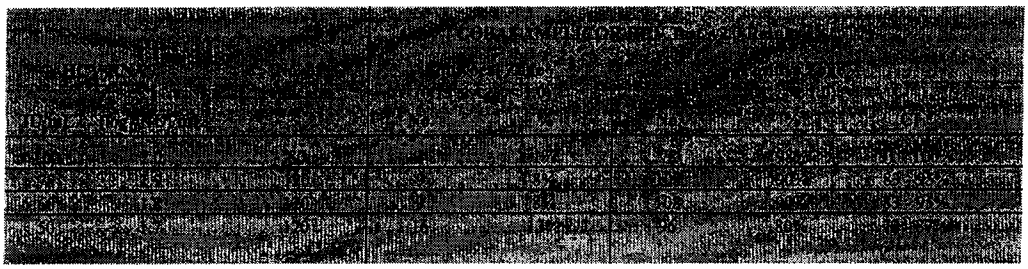
Limit of Detection (LOD)

Analytical sensitivity of the COBAS AMPLICOR HCV Test, v2.0 was determined by studying the WHO International Standard for HCV genotype 1 RNA[18]. This standard was diluted in HCV-negative serum and in HCV-negative EDTA plasma to concentrations of 100, 75, 60 and 50 IU/mL. Each concentration was tested with the COBAS AMPLICOR HCV Test, v2.0 in replicates ranging from 80 to 120 (Tables 4 and 5).

LODs were determined as the lowest HCV RNA concentration that resulted in ≥ 95% of results yielding A_{660} ≥ 1.0, the cutoff for Positive results with the COBAS AMPLICOR HCV Test, v2.0. By this criterion, the LOD for HCV RNA in serum was 100 IU/mL or 2.0 $\log_{10}$ IU/mL. The LOD for HCV RNA in EDTA plasma was 60 IU/mL or 1.8 $\log_{10}$ IU/mL (although 75 IU/mL yielded 94% Positive results, > 95% of all results were Positive for HCV RNA ≥ 60 IU/mL).

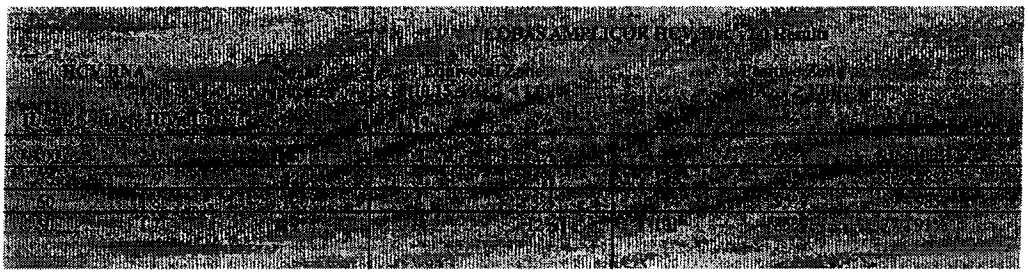
Samples yielding a result in the Equivocal Zone (EZ) were not repeat tested (per *"Interpretation of Results"* section), so a final result was not obtained for these samples. If repeat testing were done, it is reasonable to assume that frequencies of Positive results would increase, possibly resulting in lower LODs. By excluding Equivocal results, for example, the 95% threshold of Positive results was reached at 60 IU/mL, or 1.8 $\log_{10}$ IU/mL, for serum (108/111 = 97.3%). This threshold was reached at 50 IU/mL, or 1.7 $\log_{10}$ IU/mL, for EDTA plasma (101/105 = 96.2%).

Table 4
Limit of Detection in Serum, Determined with the WHO International Standard for HCV Genotype 1 RNA



* Exact 95% binomial confidence interval.

Table 5
Limit of Detection in EDTA Plasma, Determined with the WHO International Standard for HCV Genotype 1 RNA



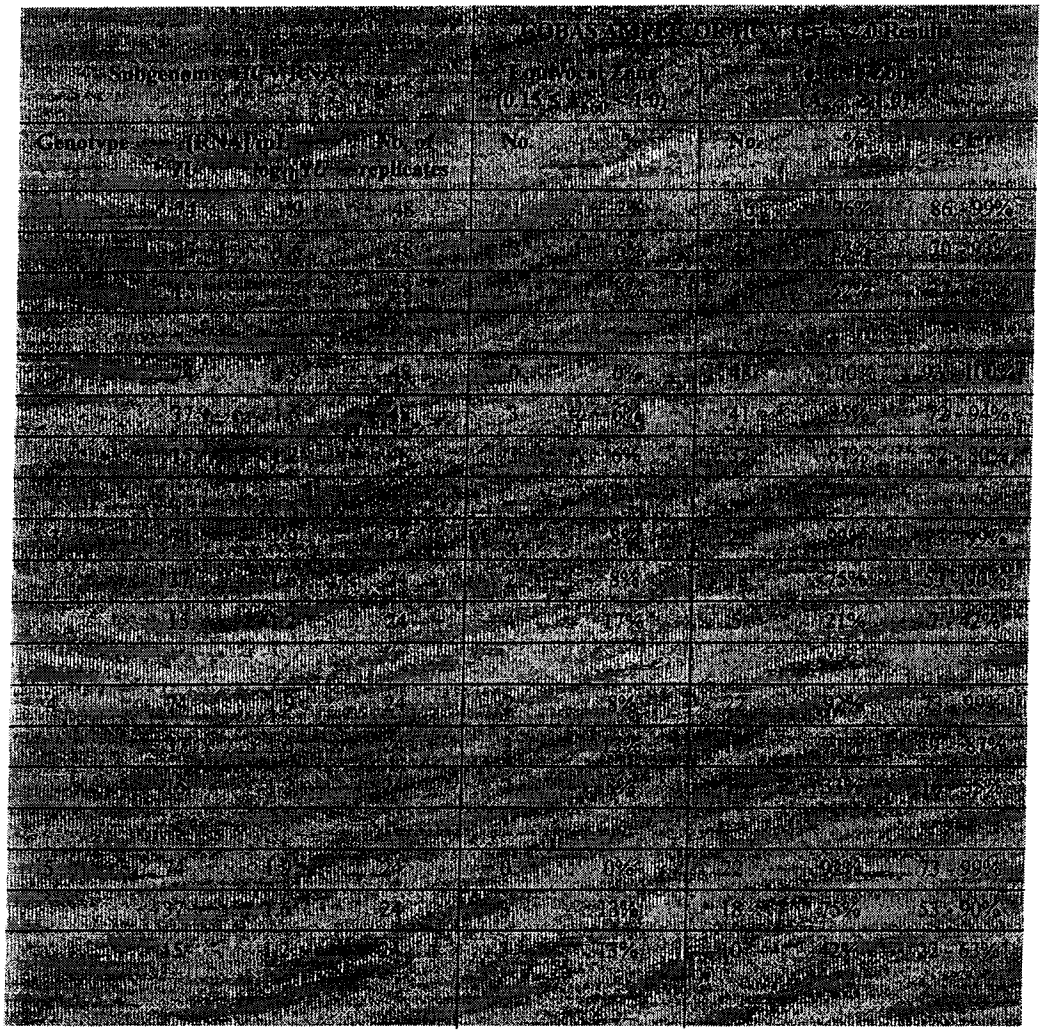
* Exact 95% binomial confidence interval.

** One replicate, yielding a Potentially Inhibited result result (A_{660} for HCV IC RNA was < 0.15) was excluded, because such results in this study were more likely to have represented loss of RNA than presence of an inhibitor.

Detection of HCV Genotypes

Quantified subgenomic RNAs, transcribed from cloned cDNAs, were used to approximate analytical sensitivity of COBAS AMPLICOR HCV Test, v2.0 for five HCV genotypes: 1, 2, 3, 4 and 5. (Genotypes of seven viruses, including two representatives each of genotypes 1 and 2, were determined by using research methods that have not been evaluated or approved by FDA; however, genotyping by many such methods is generally recognized to be accurate whereas many subtyping techniques vary in accuracy). Each RNA transcript, consisting of the 5'-untranslated and core regions, was quantified by spectrophotometry (A_{260}) and then diluted to three different concentrations in HCV Specimen Diluent, v2.0. Twenty-four replicates of each concentration were tested by the COBAS AMPLICOR HCV Test, v2.0. The number of Positive results at each concentration was determined (Table 6). Only the highest tested concentrations in this study (74 *IU*/mL) were approximately equal to the LODs with the WHO International Standard for HCV genotype 1 RNA (Tables 4 and 5); the 74 *IU*/mL genotypes 1 and 2 samples yielded ≥ 95% of Positive results. This study demonstrated, however, similar Positive result frequencies for subgenomic RNAs representing genotypes 1-5.

Table 6
Detection of Subgenomic RNAs Representing Five HCV Genotypes



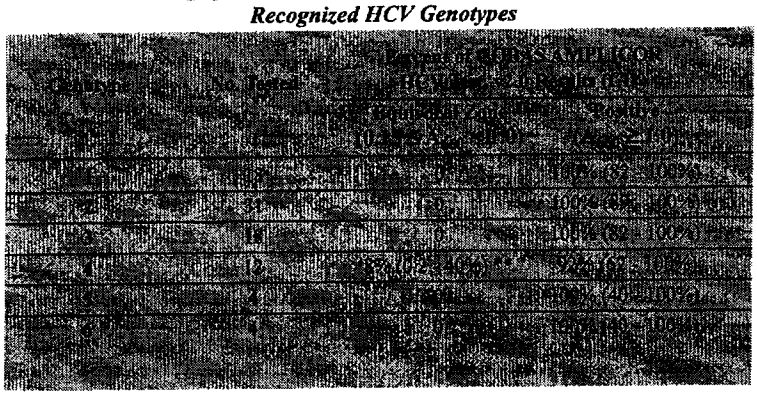
* Two different viruses were represented as subgenomic RNAs for genotypes 1 and 2, so two sets of 24 replicates were tested for each genotype. Mean amount of RNA per tested 50 µL aliquot is 1/20 of per mL concentration.

** Exact 95% binomial confidence interval.

Detection of Clinical HCV Strains, Genotypes 1-6

The COBAS AMPLICOR HCV Test, v2.0 was evaluated under research laboratory conditions to determine if it would yield Positive results with 87 clinical specimens containing HCV strains that represented the six recognized genotypes. Genotypes were identified by using research methods that have not been approved or evaluated for accuracy by FDA; however, genotyping by many such methods is generally recognized to be accurate whereas many subtyping techniques vary in accuracy. The majority of these specimens were from patients in an antiviral efficacy study and were assumed to contain HCV RNA concentrations much higher than the LOD for the COBAS AMPLICOR HCV Test, v2.0. These specimens are representative of HCV RNA concentrations in the indicated patient population. Among the 87 specimens, 86 yielded Positive results and a single genotype 4 specimen yielded an Equivocal Zone result (Table 7).

Table 7
Testing of Clinical HCV Strains Representing the Six Recognized HCV Genotypes



* Exact 95% binomial confidence intervals for % positive.

** Result from initial testing only: study design did not include repeat testing.

Analytical Specificity

Cross Contamination

Two studies were done under controlled laboratory conditions to assess the potential cross contamination rate of the COBAS AMPLICOR HCV Test, v2.0. In 25 runs of the COBAS AMPLICOR HCV Test, v2.0, multiple operators tested replicates of plasma specimens containing clinically-pertinent concentrations of HCV RNA (6.5 $\log_{10}$ *IU*/mL) that alternated with replicates of an HCV-negative specimen. Combined results indicated a cross contamination rate of 0.7% (2/273).

The potential for cross contamination was also examined in two reproducibility studies performed under clinical laboratory conditions with lower concentrations of HCV RNA. These studies demonstrated that cross contamination did not occur at [HCV RNA] = 2.3 $\log_{10}$ *IU*/mL with > 530 HCV-negative specimens (see "*Reproducibility*" section) and at [HCV RNA] ≤ 4.7 $\log_{10}$ *IU*/mL with 171 specimens.

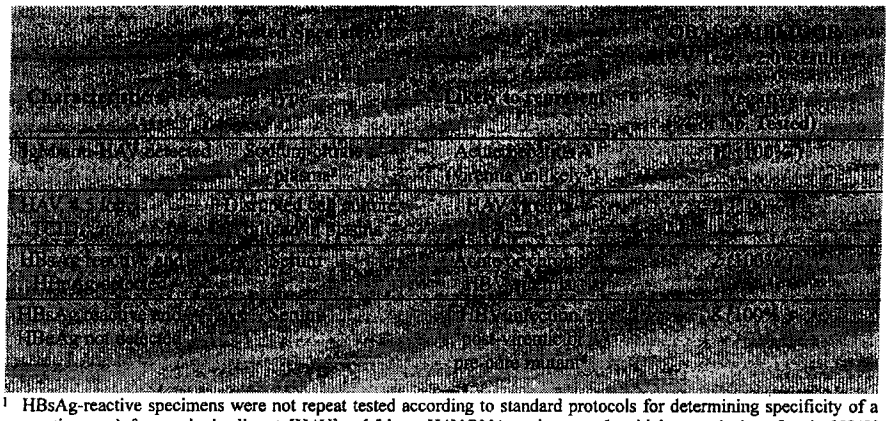
These results emphasize that, while cross contamination occurred infrequently, laboratories should carefully follow instructions in this Method Manual.

Specimens Representing Infections with HAV or HBV

In the United States, HAV, HBV and HCV are associated with the vast majority of viral hepatitis (other prevalent agents, such as Epstein-Barr virus, may induce hepatitis but usually as part of a syndrome affecting multiple organs). While HBV, like HCV, causes acute and chronic disease, HAV has not been associated with chronic infection.

Specificity of the COBAS AMPLICOR HCV Test, v2.0 was evaluated for cross-reactivity by testing specimens that represented certain manifestations of infection with HAV or HBV (Table 8). All tested serum and plasma specimens yielded Negative results.

Table 8
Testing of Specimens Representing HAV and HBV Infections



[1] HBsAg-reactive specimens were not repeat tested according to standard protocols for determining specificity of a reactive result from a single aliquot. [HAV] ≈ 6.5 $\log_{10}$ HAV RNA copies per mL, which exceeds that of typical HAV viremia; tested strain is not cytopathic and was diluted in plasma for which anticoagulant is not known.

[2] Data do not imply a claim for using plasma from blood collected in sodium citrate (for which performance has not been established with the COBAS AMPLICOR HCV Test, v2.0).

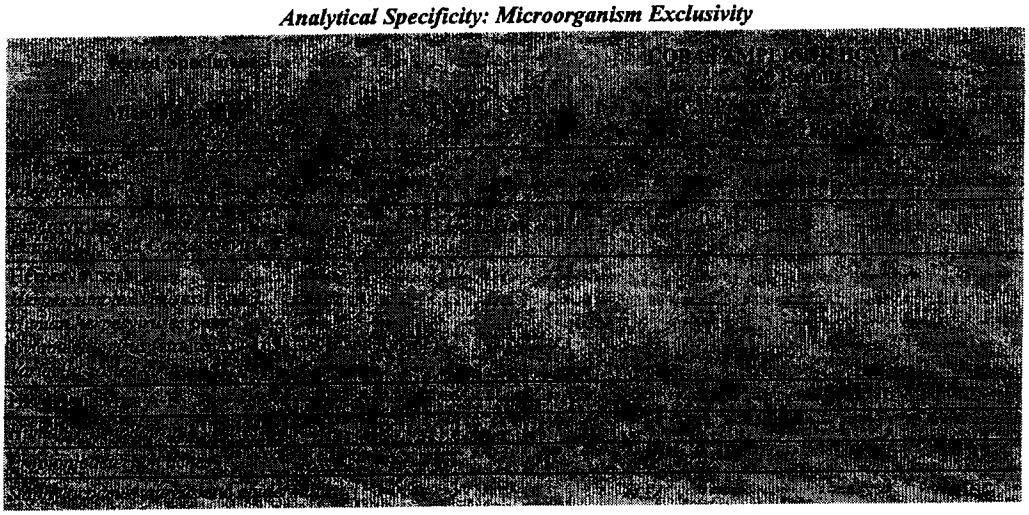
[3] Most patients with acute hepatitis A no longer release HAV from hepatocytes into blood or feces; viremia is usually during the incubation period. IgM anti-HAV can be detectable for months after the acute phase.

[4] Stage of HBV infection (if any) cannot be determined from available data.

Microorganism Exclusivity

Specificity of the COBAS AMPLICOR HCV Test, v2.0 was evaluated by testing for potential cross-reactivity with, or interference by, pathogenic microorganisms and normal epidermal microflora that could be present in specimens. Twenty-nine specimens that contained virus (25) or bacteria (4) yielded Negative HCV and Positive HCV IC results (Table 9). These results indicate that the COBAS AMPLICOR HCV Test, v2.0 did not cross-react with a variety of viruses and bacteria that could be present in specimens and that these microorganisms did not interfere with amplification of HCV IC RNA.

Table 9
Analytical Specificity: Microorganism Exclusivity



Potentially Interfering Substances, Including Inhibitors

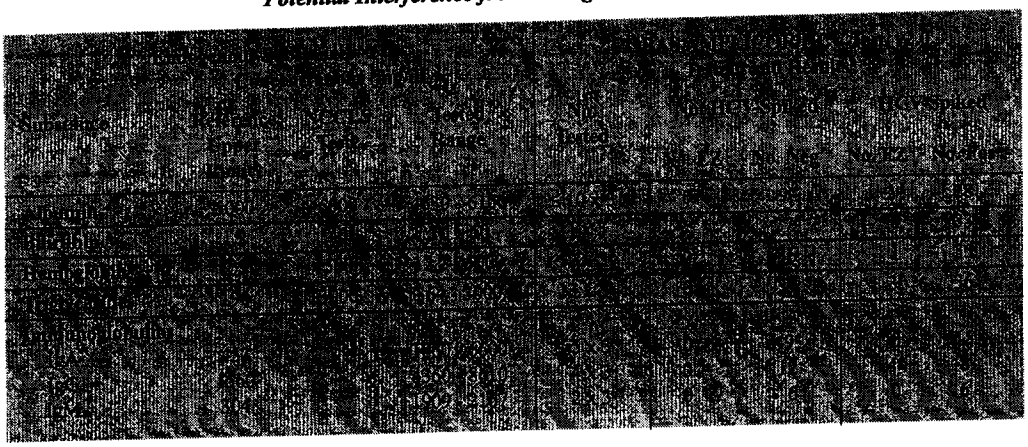
Endogenous Substances

Serum specimens containing elevated concentrations of endogenous substances were tested for interference with the COBAS AMPLICOR HCV Test, v2.0 (Table 10). These specimens were tested neat or after spiking with a near-LOD concentration of HCV RNA (100 *IU*/mL). Each specimen was tested in triplicate.

Specimens not spiked with HCV (neat) yielded Negative results, with the exception of two specimens that contained elevated levels of bilirubin. For these two specimens, it could not be determined if COBAS AMPLICOR HCV Test, v2.0 results represented accurate detection of HCV RNA (anti-HCV results were not available). The remaining elevated bilirubin specimens indicated lack of interference leading to False Positive results.

All three aliquots of an HCV-spiked high IgG (8610 mg/dL) specimen yielded Equivocal (EZ) results. For one other HCV-spiked specimen, one of the three aliquots yielded an Equivocal result. These results indicate that several of the tested substances might interfere with the COBAS AMPLICOR HCV Test, v2.0, particularly IgG (4 of 12 tested aliquots) or triglycerides (3 of 39 aliquots, representing 3 specimens). Any conclusions about these results should, however, be tentative because available data are limited for these and other such specimens, and because Equivocal results are expected for several percent of aliquots when [HCV RNA] is near-LOD (see *"Analytical Sensitivity"* and *"Reproducibility"* sections).

Table 10
Potential Interference from Endogenous Substances



[a] Three aliquots of each specimen were tested; EZ, Equivocal Zone ($0.15 \leq A_{660} < 1.0$); Neg, $A_{660} < 0.15$; Pos, $A_{660} \geq 1.0$.

[b] NCCLS Document EP7-P, Vol. 6, No. 13 "Interference Testing in Clinical Chemistry" Appendix A,"Recommended Serum/Plasma Test Levels, II. Endogenous Substances," pages 326 - 372. Reference upper limits for Immunoglobulins are for adults at the Johns Hopkins Medical Institutions, Baltimore, MD.

[c] Only three specimens were tested because of volume limitations

[d] One Equivocal result for each of two specimens

[e] Two Equivocal and one Positive result for one specimen; three Positive results for another specimen

[f] One Equivocal result for each of three specimens

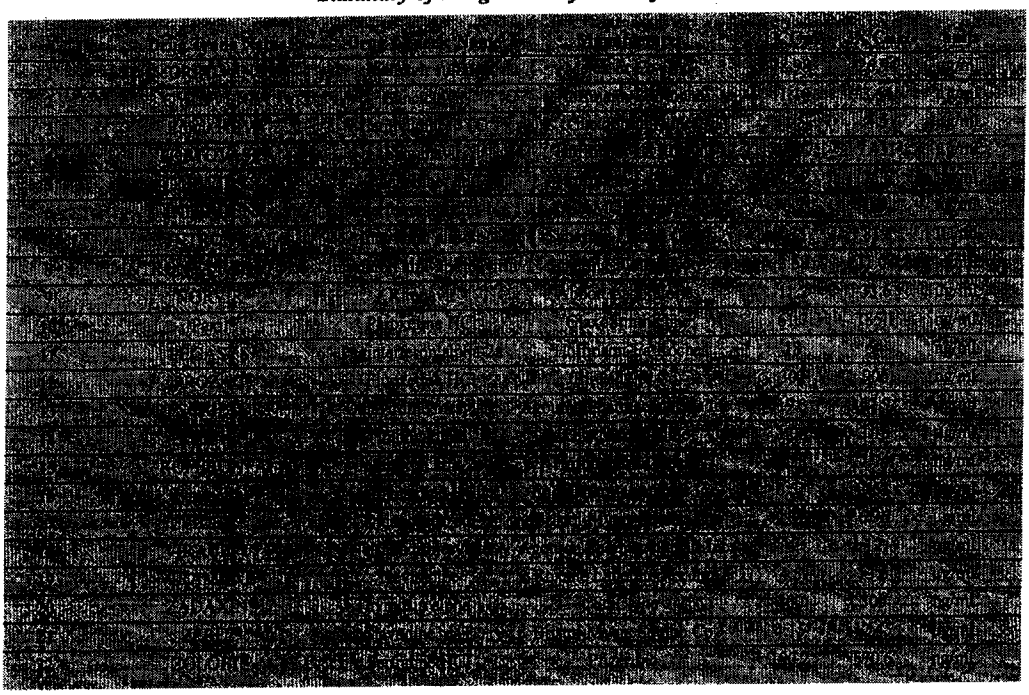
[g] Three Equivocal results for one specimen; one Equivocal result for another specimen

Therapeutic Drugs

Drugs for infectious diseases, or for several conditions associated with hepatitis C therapy, were evaluated for their potential to interfere with the COBAS AMPLICOR HCV Test, v2.0. Among the former were drugs for hepatitis C, hepatitis B, HIV, influenza A, or cytomegalovirus-associated syndromes. Each drug was spiked to two plasma concentrations, peak and 3-times peak (1X and 3X C_{max}), into plasma that contained HCV at near-LOD 100 *IU*/mL or was HCV RNA-negative (Table 11). Specimens were tested in triplicate, with and without spiked drugs.

Evaluated drugs did not yield False Positive (no HCV, but $A_{660} \geq 1.0$) or False Negative (HCV present, but $A_{660} < 0.15$) results. At each drug concentration, all HCV-positive specimens yielded Positive results except for one aliquot that contained Crixivan at 1X C_{max} that yielded an Equivocal result. Thus, these drugs did not appear to interfere with the ability of COBAS AMPLICOR HCV Test, v2.0 to detect HCV RNA.

Table 11
Summary of Drugs Tested for Interference



Co-infections

Additional testing for interference with the COBAS AMPLICOR HCV Test, v2.0 was performed by using serum specimens from 25 patients who were likely to have had an active HCV infection, or to have cleared HCV, and to have been actively infected with HBV, HIV or both HBV and HIV (Table 12). Many of these patients had hemophilia or were injection drug users, placing them at high risk of infection with all of these viruses (the others were categorized as "high risk" without further definition). Two of the patients with presumptive evidence of anti-HCV and HBsAg also had evidence of past or current infection with HAV, or of successful vaccination against HAV: Positive results in an assay for "total" (IgG and IgM) antibodies to HAV.

Among these 25 specimens, 21 yielded Positive results with the COBAS AMPLICOR HCV Test, v2.0, and 4 yielded Negative results (with HCV IC results that did not indicate inhibition). The 4 Negative results were all from specimens with presumptive evidence of active HBV replication (HBsAg, single-aliquot reactive); one of the specimens was also anti-HIV reactive and two were specimens that were also total anti-HAV positive.

Data from the samples with presumptive HIV/HCV infections enable a tentative conclusion that HIV was unlikely to interfere with the COBAS AMPLICOR HCV Test, v2.0. Negative results (one HBV/HCV, two HCV/HBV/HAV and one HBV/HIV) might indicate (i) HCV clearance, (ii) loss of HCV RNA during inadequate specimen transport or storage, (iii) HCV RNA concentration below the LOD for the COBAS AMPLICOR HVC Test, v2.0 or (iv) test interference from HBV or antibodies to HAV. Most of the data demonstrated no interference from these co-infections.

Table 12
Testing of Serum Specimens Representing Possible Co-Infections

* Serologic testing by EIA: R, reactive (single aliquot); NR, non-reactive; NT, not tested. Although reactive specimens were not repeat tested according to standard protocols for determining specificity of reactive antibody or antigen results from a single aliquot, other information about subjects supported accuracy of anti-HIV results and was consistent with accuracy of anti-HCV and HBsAg results.

Clinical Evaluation

Clinical Study Objectives and Methods

A prospective study was conducted at four U.S. hepatology centers to evaluate clinical utility of the COBAS AMPLICOR HCV Test, v2.0 for diagnosis of active HCV infection in patients with biochemical, clinical and/or histological evidence of liver disease. Test performance was evaluated against two standards:

- Anti-HCV serology
- A combination of anti-HCV serology, serum ALT levels and histological findings in liver tissue.

Studied patients were being investigated for HCV infection and/or liver disease, and some had previously been treated for chronic hepatitis C*. Patients were excluded if they had undergone liver transplantation or received antiviral therapy for hepatitis C within 6 months of study screening.

The physicians evaluating patients recorded clinical diagnoses (chronic HCV infection, alcoholic liver disease, primary biliary cirrhosis, etc.) based on history, physical examination and laboratory results available prior to enrollment. In many cases, the investigators had access to anti-HCV results but they were blinded to HCV RNA results. For a subset of the patients, liver histology had been characterized in the past; these findings were categorized according to evidence of hepatitis (see below, *"Clinical Performance Compared to ...Histological Findings"*).

* Study included patients for whom COBAS AMPLICOR HCV Test, v2.0 is not indicated. Previously treated patients were included because those with active infections were considered to be virologically representative for determining COBAS AMPLICOR HCV Test, v2.0 performance; however, their data do not imply performance for monitoring HCV-associated disease or response to treatment. Other patients, with negative anti-HCV results and/or "normal" (within reference range) ALT levels and no histological evidence of hepatitis, were studied for approximating specificity of this test, but these data do not imply performance for testing of patients without liver disease or antibody evidence of HCV infections (see Warnings in *"Intended Use"* section).

Serum and/or EDTA plasma specimens were collected after enrollment. Serum ALT was quantified to provide biochemical evidence of liver disease. These serum and EDTA plasma specimens were tested for antibody and RNA evidence of HCV infection. Anti-HCV testing was by EIA (version 3.0 at one study site and version 2.0 at the others). Specimens that were repeatedly EIA-reactive (EIA-RR) were also tested by strip immunoassay (SIA, v2.0). EIA and SIA testing and interpretation followed manufacturers' package insert instructions. HCV RNA results from the COBAS AMPLICOR HCV Test, v2.0 were interpreted per this Method Manual.

Performance of the COBAS AMPLICOR HCV Test, v2.0 was determined by comparison to anti-HCV results. For the subset of patients for whom a liver histology report was available, COBAS AMPLICOR HCV Test, v2.0 performance was further evaluated against anti-HCV serology, serum ALT levels and histological findings.

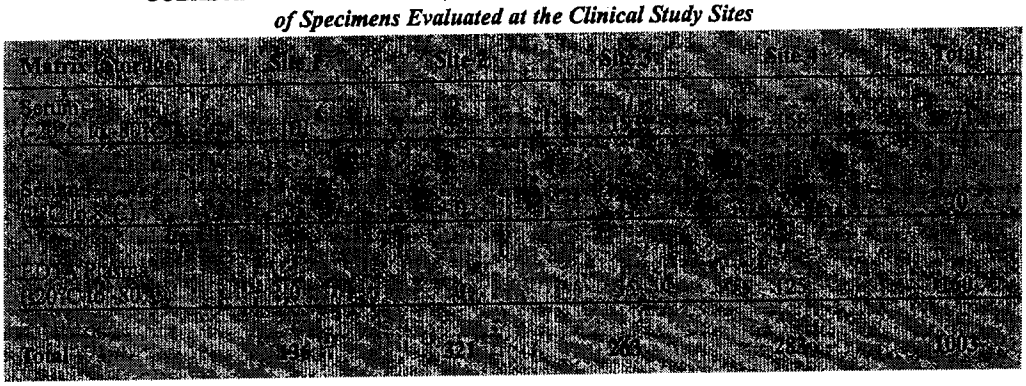
Clinical Study Results

A total of 1,003 specimens were evaluated from 848 patients. Their mean age was 46 years; 55% were male; and 72%, 13%, 11% and 2% were, respectively, Caucasian, African-American, Hispanic and Asian. Reasons for attendance at the hepatology clinics included prior HCV diagnosis (40%) and evaluation of HCV (35%) or liver disease (26%).

Clinical diagnoses at enrollment included chronic HCV infection (73%), autoimmune hepatitis (2%), alcoholic liver disease (6%), chronic HBV infection (4%), and primary biliary cirrhosis (5%); 14% had other diagnoses. These patients' mean ALT value was 96 IU/mL (range, 7-668); 53% had a liver histology report and 18% had been treated for hepatitis C.

The number, types and storage conditions of specimens are summarized in Table 13.

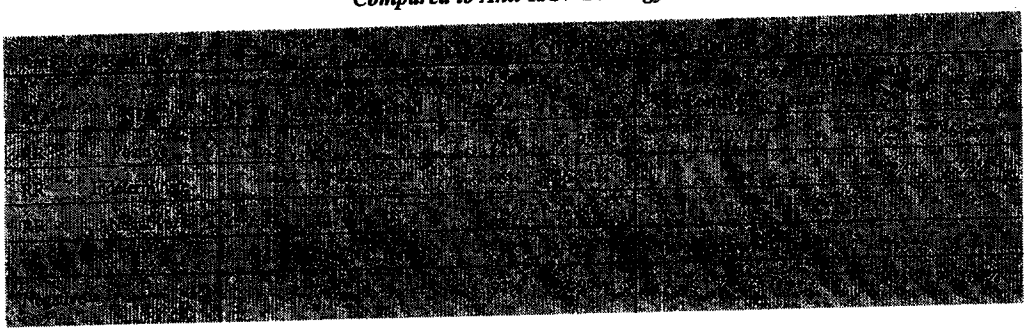
Table 13
COBAS AMPLICOR HCV Test, v2.0: Number, Types and Storage Conditions of Specimens Evaluated at the Clinical Study Sites



Clinical Performance Compared to Anti-HCV Serology

Performance of the COBAS AMPLICOR HCV Test, v2.0 as determined by comparison to anti-HCV findings, was similar across the four study sites. Table 14 summarizes these data for all sites, patients and specimen types.

Table 14
COBAS AMPLICOR HCV Test, v2.0 Performance with Serum and EDTA Plasma Specimens, Compared to Anti-HCV Serology[1]



[1] Specimens were from 848 patients, including 154 who had received antiviral therapy for chronic hepatitis C that stopped > 6 months before collecting specimens. While these previously treated patients were included because those with active infections were considered to be virologically representative for determining test performance, their data do not imply performance for monitoring HCV-associated disease or response to treatment. The 694 untreated patients included >200 who had negative anti-HCV EIA results and/or ALT concentration within reference range and no histological evidence of hepatitis; they were studied for approximating test specificity, but their data do not imply performance for testing of individuals without liver disease or antibody evidence of HCV infections (see Warnings in *"Intended Use"* section). In particular, Positive COBAS AMPLICOR HCV Test, v2.0 results were obtained for three patients who had liver disease other than hepatitis C and for whom there was no evidence, or insufficient evidence, to conclude that they were actively infected with HCV.

[2] Anti-HCV testing by version 2 or version 3 EIA (enzyme immunoassay; RR, repeatedly reactive) and by version 2 SIA (strip immunoassay). Among sera, 75% were repeatedly reactive by anti-HCV EIA (EIA-RR); 71%, 3.5% and 0.1% were respectively also positive, indeterminate and negative by SIA; 84% of EDTA plasma specimens were EIA-RR (82% were also SIA-positive and < 3% were SIA-indeterminate).

[3] Results include final interpretations of Potentially Inhibited and Equivocal initial testing result. Eleven serum specimens were excluded from this analysis because quantities were not sufficient for appropriate repeat testing (see *"Interpretation of Results"* section). CI, 95% confidence intervals by exact binomial method.

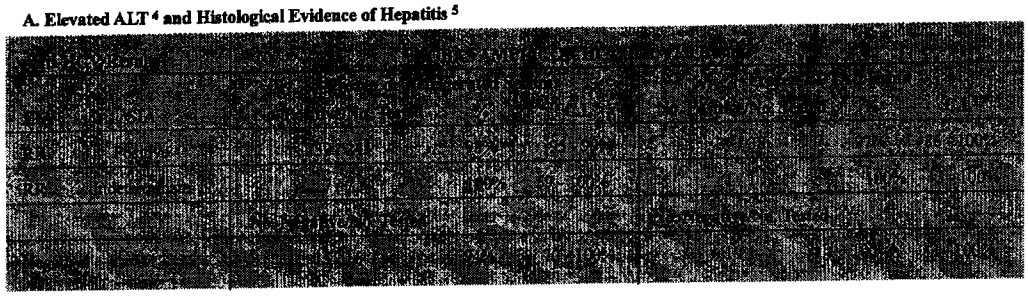
HCV RNA was detected in the vast majority of SIA-positive specimens for both matrices (94% and 92%). HCV RNA was also detected in 66% and 50% of serum and EDTA plasma samples, respectively, from patients with indeterminate SIA results. HCV RNA was not detected in the vast majority of EIA-negative samples for both matrices (97% and 96%). Thus, there was good correspondence between COBAS AMPLICOR HCV Test, v2.0 results and anti-HCV results for serum and EDTA plasma.

Clinical Performance Compared to Anti-HCV, Biochemical and Histological Findings

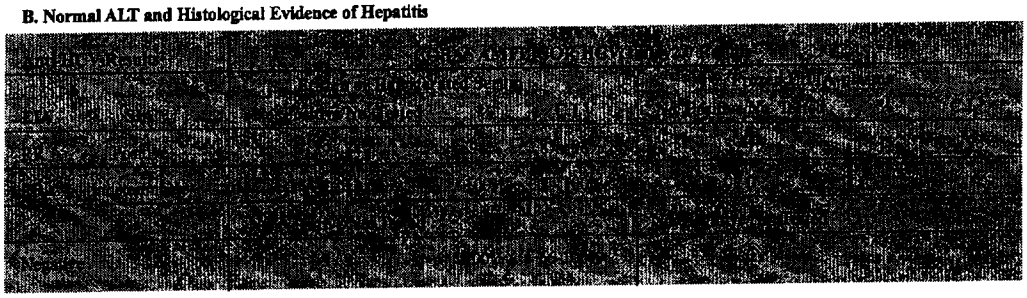
Performance of the COBAS AMPLICOR HCV Test, v2.0 was further evaluated against anti-HCV test results, ALT concentrations and histological findings in the subset of patients from whom liver tissue had been collected in the past. Intervals varied between collection of liver tissue and collection of blood for HCV RNA testing, so histological findings may not have represented disease activity when blood was collected for the COBAS AMPLICOR HCV Test, v2.0, anti-HCV testing and ALT determination. A physician at Roche Molecular Systems categorized findings as histological evidence of hepatitis when the inflammatory infiltrate and pattern of necrosis were consistent with chronic hepatitis; many such specimens also had fibrosis or cirrhosis. Data comparing performance of the COBAS AMPLICOR HCV Test, v2.0 to anti-HCV, biochemical and histological findings are shown in Table 15.

Table 15
Performance of the COBAS AMPLICOR HCV Test, v2.0 with Serum and EDTA Plasma Specimens, Compared to Anti-HCV, Biochemical and Histological Findings[1]

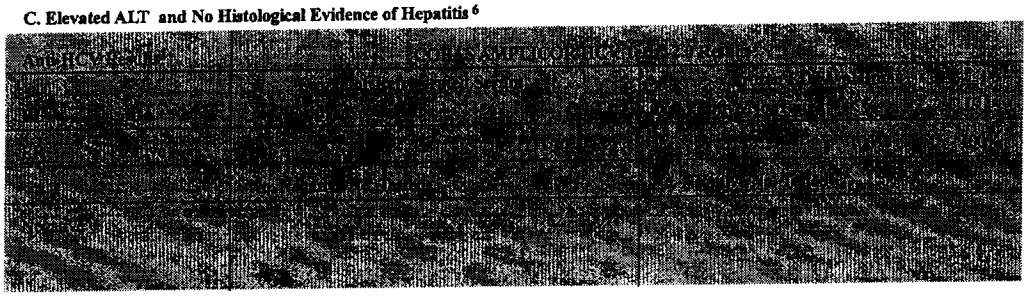
A. Elevated ALT [4] and Histological Evidence of Hepatitis [5]



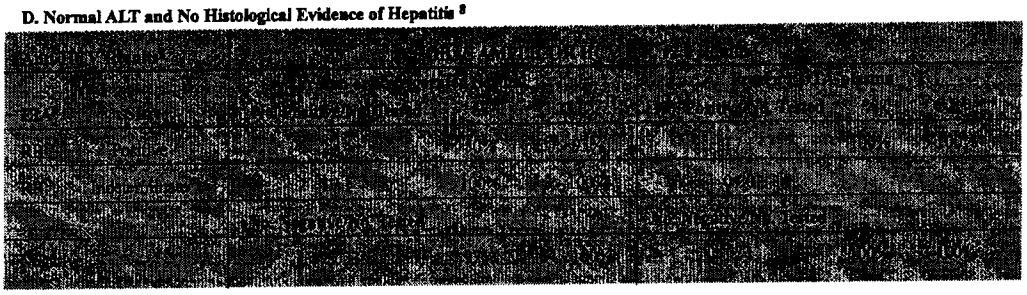
B. Normal ALT and Histological Evidence of Hepatitis



C. Elevated ALT and No Histological Evidence of Hepatitis [6]



D. Normal ALT and No Histological Evidence of Hepatitis [8]



[1] Specimens were from untreated patients and from patients who had received antiviral therapy for chronic hepatitis C that stopped > 6 months before study specimens were collected. While previously treated patients were included because specimens from those with active HCV infections were considered to be virologically representative for determining COBAS AMPLICOR HCV Test, v2.0 performance, those data do not imply performance for monitoring HCV-associated disease or response to treatment: see Warnings in *"Intended Use"* section. Similarly, patients who had anti-HCV EIA negative results were studied for approximating the specificity of the COBAS AMPLICOR HCV Test, v2.0 but these data do not imply performance for testing of anti-HCV EIA negative individuals: see Warnings in *"Intended Use"* section.

[2] Anti-HCV testing by version 2 or version 3 EIA (enzyme immunoassay; RR, repeatedly reactive) and by version 2 SIA; strip immunoassay.

[3] Results include final interpretations of Potentially Inhibited and Equivocal testing result. CI, 95% confidence intervals by exact binomial method.

[4] ALT, alanine aminotransferase; elevated, ALT concentration > upper limit of the site's reference range; normal, within reference range

[5] Forms of hepatitis included hepatitis C, hepatitis B, autoimmune hepatitis and others

[6] Included histologically normal tissue, or non-specific inflammatory changes or evidence of other liver disease (such as primary biliary cirrhosis)

[7] No SIA-indeterminate results among these specimens

[8] Patients who had normal ALT levels and no histological evidence of hepatitis were studied for approximating the specificity of the COBAS AMPLICOR HCV Test, v2.0 but these data do not imply performance for testing of such individuals: see Warnings in the *"Intended Use"* section.

Elevated ALT level and histological evidence of hepatitis (Table 15.A). Among anti-HCV EIA-RR/SIA-positive specimens, COBAS AMPLICOR HCV Test, v2.0 results were respectively Positive for 98% and 97% of serum and EDTA plasma specimens and in all 8 EIA-RR/SIA-indeterminate specimens. Negative results were obtained with 16 out of 17 specimens from EIA-negative patients; their histological characteristics were suggestive of autoimmune hepatitis, hepatitis B or other forms of non-C hepatitis.

ALT level within reference range and histological evidence of hepatitis (Table 15.B). COBAS AMPLICOR HCV Test, v2.0 results were respectively Positive for 94% of serum and 87% of EDTA plasma specimens among EIA-RR/SIA-positive specimens and in one SIA-indeterminate specimen. COBAS AMPLICOR HCV Test, v2.0 results were Negative for all 9 EIA-negative sera from patients with histological features of hepatitis that were suggestive of diseases other than hepatitis C.

Elevated ALT level and no histological evidence of hepatitis (Table 15.C). Among EIA-RR/SIA-positive patients, COBAS AMPLICOR HCV Test, v2.0 results were Positive for 5 of the 7 of serum specimens and in both EDTA plasma specimens. COBAS AMPLICOR HCV Test, v2.0 results were Negative for 97% of EIA-negative serum specimens.

ALT level within reference range and no histological evidence of hepatitis (Table 15.D). COBAS AMPLICOR HCV Test, v2.0 results were Positive for two of the four sera and the single EDTA plasma specimen with EIA-RR/SIA-positive results, and in the single EIA-RR/SIA-indeterminate specimen. COBAS AMPLICOR HCV Test, v2.0 results were Negative for 97% of serum specimens and in the one EDTA plasma specimen from EIA-negative patients. Histological liver disease was evident in these cases (but without features of hepatitis).

Summary and Conclusions

Clinical evaluation of the COBAS AMPLICOR HCV Test, v2.0 demonstrated that this test's results, for both serum and EDTA plasma, were highly correlated with anti-HCV testing results. There was also a high degree of concordance between COBAS AMPLICOR HCV Test, v2.0 results and serologic, biochemical and histological findings. The study demonstrated clinical utility for COBAS AMPLICOR HCV Test, v2.0 for diagnosis of active HCV infection among patients who had evidence of liver disease and antibody evidence of infection with HCV.

Reproducibility

To evaluate reproducibility of the performance of COBAS AMPLICOR HCV Test, v2.0, two six-member sample panels were studied, one prepared in frozen serum and the other in frozen EDTA plasma. Four HCV-positive samples were prepared as dilutions from three genotype 1 clinical HCV specimens, to yield nominal HCV RNA concentrations of 50, 75, 100 and 200 *IU*/mL. The remaining two samples did not contain detectable HCV RNA. Reproducibility was evaluated across study sites, days and reagent lots. Each matrix was tested by two operators at each of three sites (two clinical reference laboratories and a Roche Molecular Systems laboratory). Each operator conducted 5 days of testing on each of three reagent lots. To assess within-day performance, three aliquots of each sample were tested within each run.

The 3209 results from this study are summarized in Table 16 for serum and Table 17 for EDTA plasma samples. Negative results were obtained with 100% of the > 500 HCV RNA-negative samples of each matrix, for overall results and for each within-study variable (Tables 16.A, 16.C, 17.A and 17.C). This study demonstrated complete reproducibility of qualitative results with HCV RNA-negative samples.

Reproducibility for samples containing HCV RNA was also assessed by comparing frequencies of Positive results for within-study variables (Tables 16.B and 17.B). For each HCV RNA concentration, all confidence intervals overlapped for each within-study variable (see second footnote to Table 16 and to Table 17). Therefore, the observed differences between any Positive result frequencies, within a variable for a particular HCV RNA concentration, are probably due to chance. These results demonstrate reproducibility with samples containing HCV RNA.

Several trends are worth noting. For serum samples with the highest concentration (200 *IU*/mL) of HCV RNA, the ranges of Positive result frequencies were 99-100% for Site-to-Site and Lot-to-Lot and 98-100% for Day-to-Day; these represent the narrowest ranges of Positive result frequencies (highest reproducibility) among serum samples. For serum samples with 50 *IU*/mL, the ranges of Positive result frequencies were 85-89% for Site-to-Site, 78-91% for Lot-to-Lot and 80-93% for Day-to-Day. The latter two ranges represent the broadest ranges of Positive result frequencies (lowest reproducibility) among all samples and these were obtained with the lowest concentration of HCV RNA. Similarly, reproducibility among EDTA plasma samples varied with HCV RNA concentration. For each HCV RNA concentration in serum, the Site-to-Site range of Positive result frequencies was less broad (suggesting higher reproducibility) than those for Lot-to-Lot or Day-to-Day.

These data demonstrate reproducible performance of the COBAS AMPLICOR HCV Test, v2.0 assay across reagent lots, study sites, days and sample matrices.

Notes on Limits of Detection (LODs)

Data from this reproducibility study can also be used to calculate LODs, as determined at different laboratories, by analyzing overall and individual site results for the lowest HCV RNA concentrations that yielded ≥ 95% Positive results. For serum samples, this concentration was 100 *IU*/mL (2 $\log_{10}$ *IU*/mL) for overall results and for sites B and C (Tables 16.A and 16.B). Site A had 94% Positive results for 100 *IU*/mL but 95% of its results were Positive for HCV RNA at 75 IU/mL. These LODs are very similar to that of 100 IU/mL, determined with the WHO International Standard for HCV genotype 1 RNA under controlled laboratory conditions (Table 4).

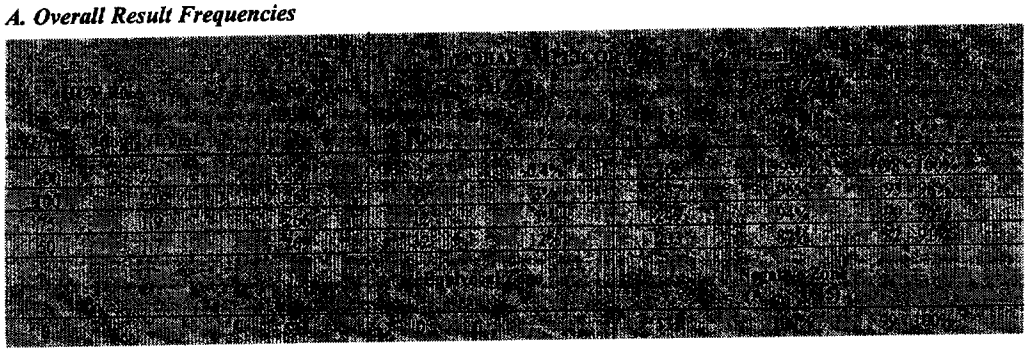
For EDTA plasma, the lowest HCV RNA concentration that yielded ≥ 95% Positive results was 75 *IU*/mL (1.9 $\log_{10}$ IU/mL) for overall results and for sites C and D (Tables 17.A and 17.B). Site A had 94% Positive results for 100 *IU*/mL but 98% of its results were Positive for HCV RNA at 75 *IU*/mL. These LODs are very similar to that of 60 IU/mL, determined with the WHO Standard under controlled laboratory conditions (Table 5).

Reproducibility-study samples yielding an Equivocal (EZ) result were not repeat tested (per *"Interpretation of Results"* section), so a final result was not obtained for these samples. If repeat testing were done, it is reasonable to assume that frequencies of Positive results would increase. By excluding Equivocal results, for example, the 95% threshold of Positive results was reached at 50 *IU*/mL, or 1.8 $\log_{10}$ *IU*/mL, for serum and for EDTA plasma. These findings are similar to those obtained by performing the same calculation on data from the LOD study with the WHO Standard (see *"Analytical Sensitivity"* section).

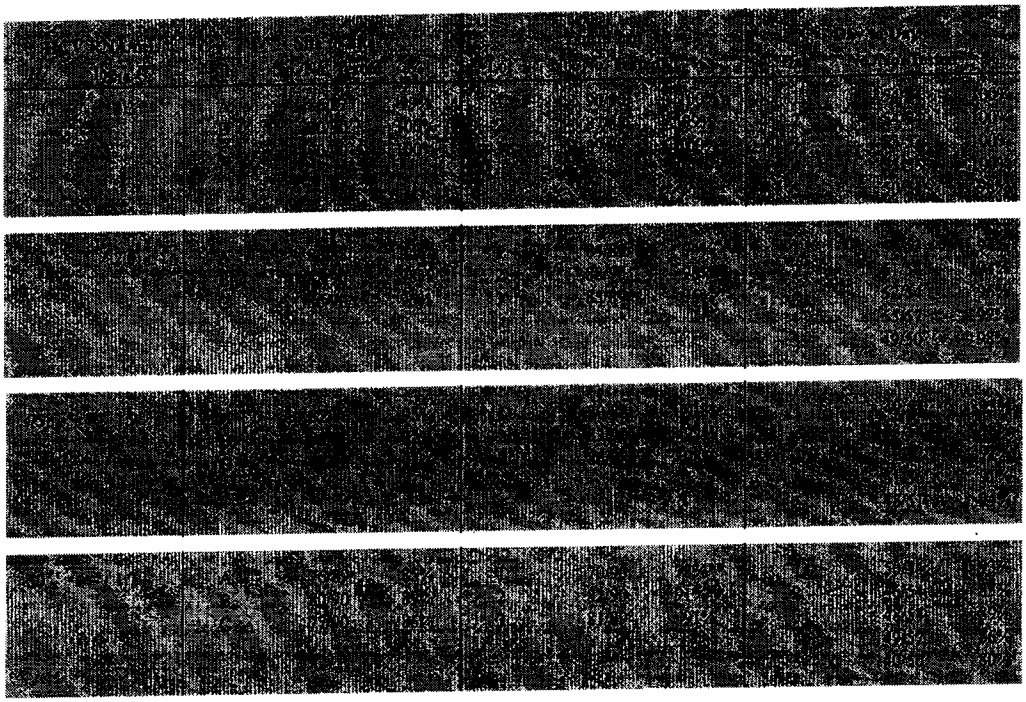
The COBAS AMPLICOR HCV Test, v2.0 usually yielded ≥ 95% Positive results for lower concentrations of tested genotype 1 HCVs in EDTA plasma than in serum (Tables 4, 5, 16, and 17). However, the inter-matrix differences were small (≤ 0.3 $\log_{10}$ *IU*/mL) and not likely to affect the indication for use (see *"Intended Use," "Expected Results,"* and *"Clinical Evaluation"* sections).

Table 16
Reproducibility: Serum Samples

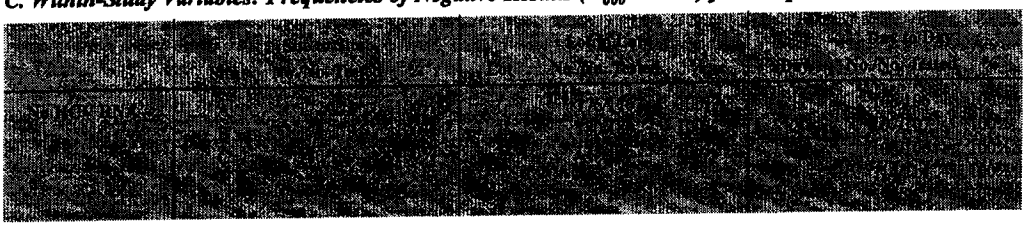
A. Overall Result Frequencies



B. Within-Study Variables: Frequencies of Positive Results (A_{660} ≥ 1.0) for Samples Containing HCV RNA*



C. Within-Study Variables: Frequencies of Negative Results (A_{660} < 0.15) for Samples Without HCV RNA

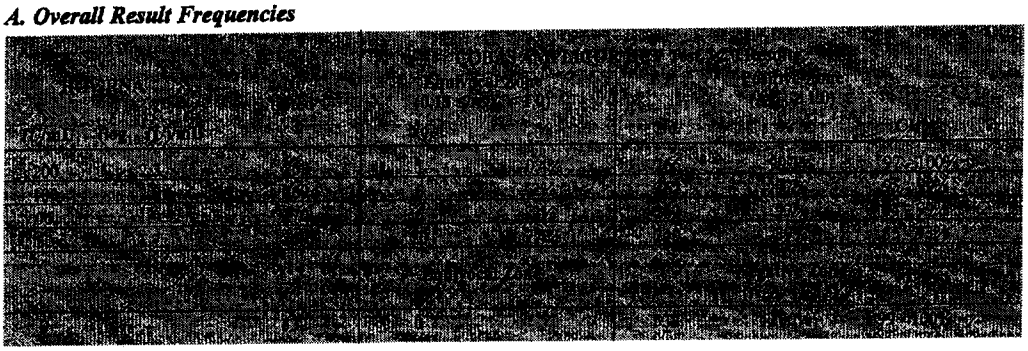


* Equivocal results were included in the denominator (No. Tested) for these calculations, but Potentially Inhibited results were excluded.

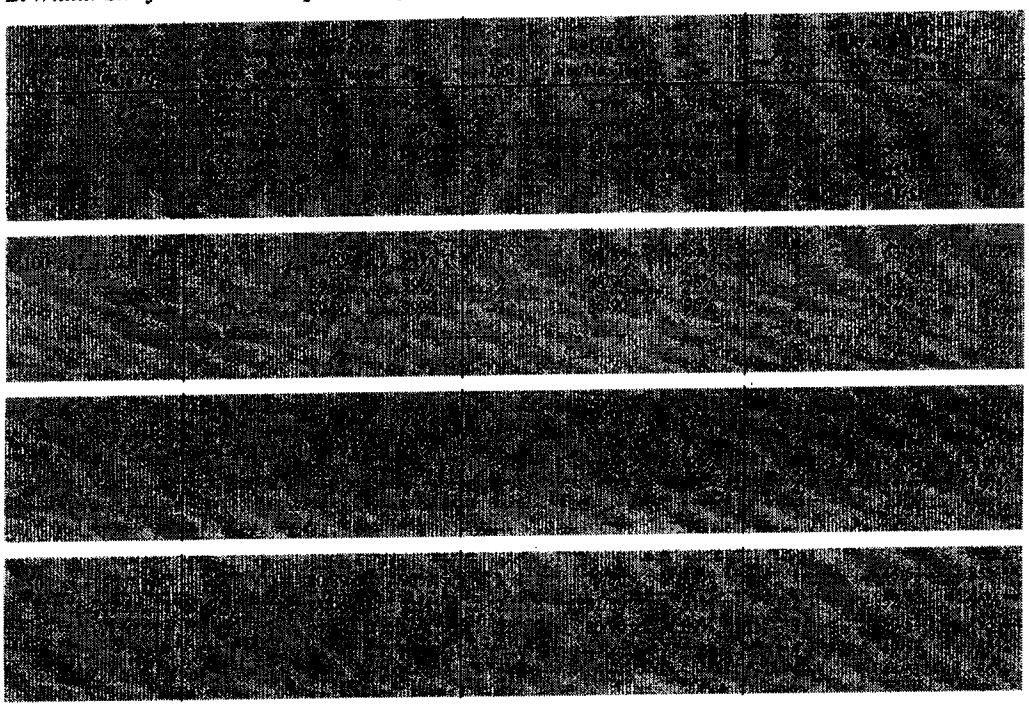
** Exact 95% binomial confidence interval calculated in B. only for the two most different frequencies for a variable (50 IU/mL, Lot-to-Lot): Lot 1 CI = 68-86% and Lot 2 CI = 83-96%; all analogous confidence intervals also overlap.

Table 17
Reproducibility: EDTA Plasma Samples

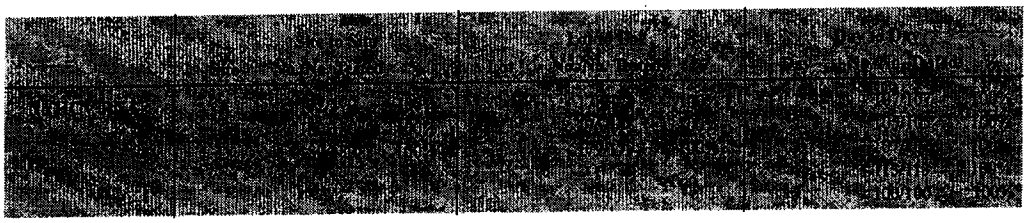
A. Overall Result Frequencies



B. Within-Study Variables: Frequencies of Positive Results ($A_{660} \geq 1.0$)* for Samples Containing HCV RNA



C. Within-Study Variables: Frequencies of Negative Results ($A_{660} < 0.15$) for Samples Without HCV RNA



* Equivocal results were included in the denominator (No. Tested) for these calculations, but Potentially Inhibited results were excluded.

** Exact 95% binomial confidence interval calculated in B. only for the two most different frequencies for a variable (50 IU/mL, Day-to-Day): Day 1 CI = 72-93% and Day 2 CI = 87-100%; all analogous confidence intervals also overlap.

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